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Spinal muscular atrophy: the role of SMN in axonal mRNA regulation

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Abstract

Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by homozygous mutations or deletions in the survival of motor neuron (*SMN1*) gene, encoding the ubiquitously expressed SMN protein. SMN associates with different proteins (Gemins 2-8, Unrip) to form a multimeric complex involved in the assembly of small nuclear ribonucleoprotein complexes (snRNPs). Since this activity is essential for the survival of all cell types, it still remains unclear why motor neurons are selectively vulnerable to low levels of SMN protein.

Aside from its housekeeping role in the assembly of snRNPs, additional functions of SMN have been proposed. The well-documented localization of SMN in axonal transport granules and its interaction with numerous mRNA-binding proteins not involved in splicing regulation suggest a role in axonal RNA metabolism.

This review will focus on the neuropathological and experimental evidence supporting a role for SMN in regulating the assembly, localization, or stability of axonal messenger ribonucleoprotein complexes (mRNPs). Furthermore, how defects in this non-canonical SMN function may contribute to the motor neuron pathology observed in SMA will be discussed.

Introduction

Spinal muscular atrophy (SMA), one of the leading genetic causes of infant mortality, is a neuromuscular disease characterized by the specific degeneration of spinal motor neurons (Pearn, 1978; Van Meerbeke and Sumner, 2011). In the vast majority of cases, SMA is caused by low levels of the survival of motor neuron (SMN) protein, due to deletions or, less frequently, mutations in the *SMN1* gene (Lefebvre et al., 1995). In humans, a duplicated form of *SMN1* exists, *SMN2*. The two genes are in large part identical except for a few nucleotide changes. One of these, located in exon 7, impairs the correct splicing of *SMN2* pre-mRNA, leading to the skipping of exon 7 and to the translation of the truncated and unstable SMN Δ 7 protein (Lorson et al., 1998; Lorson et al., 1999). Approximately 10% of the *SMN2* transcript gives rise to full length SMN protein, allowing most cells to function

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normally. However, such low levels of SMN are not sufficient to sustain the survival and function of spinal motor neurons, thus leading to progressive muscle denervation, axonal degeneration, and to the eventual loss of spinal motor neurons.

The reason of the specific vulnerability of motor neurons to low levels of SMN is still largely unknown. SMN protein is localized to both the nucleus and cytoplasm of all cell types, commonly as part of a multiprotein complex comprising Unrip and seven Gemin proteins (Gemin 2-8). The interaction map of the complex has been established, but the exact stoichiometry is still to be determined (Grimmler et al., 2005; Otter et al., 2007). The SMN complex has an essential housekeeping role in all cells to facilitate the assembly of small nuclear ribonucleoproteins (snRNPs), the key components of spliceosomes. In recent years considerable progress has been made in characterizing the function of the SMN-Gemin complex in this process, and evidence for defective snRNP assembly and pre-mRNA splicing have been observed in SMA animal models (Gabanella et al., 2007; Zhang et al., 2008). Despite this progress, it is still not clear whether the motor neuron degeneration observed in SMA is directly caused by defects in snRNP biogenesis (Kolb and Kissel, 2011). Work from the Pellizzoni laboratory suggests that defects in snRNP assembly and function may preferentially affect transcripts that are necessary for motor neuron activity (Gabanella et al., 2007), although what these mRNAs are and whether they are localized to motor axons still remains to be determined. Of note, the snRNP assembly activity in spinal cord extracts from the most severe *Smn*^{-/-}, *hSMN2* and the milder *SMNΔ7* mouse models was indistinguishable (Gabanella et al., 2007). This suggests that the difference in life span of these mice may be caused by defects in an additional function of SMN. Indeed, work from several laboratories has provided evidence for an additional function of SMN in the axon of neurons. Our review will summarize the most recent findings supporting a non-canonical role for SMN in the maintenance of motor neurons via regulating the fate of cytoplasmic and axonal mRNAs. As motor neurons have very long axons and highly specialized endplates, SMN levels might be more critical for mRNP localization in these cells. This might explain the dying-back axonopathy and specific defects in motor neuron maintenance observed in SMA.

SMA as an axonopathy

SMA is characterized by spinal motor neuron degeneration, leading to progressive denervation of skeletal and intercostal muscles, muscle weakness, paralysis, and eventual death due to respiratory failure (Crawford and Pardo, 1996). To study the pathomechanisms of this devastating disease, a large number of invertebrate animal and mouse models have been generated (for detailed reviews, see Grice et al., 2011; Park et al., 2010; Sleight et al., 2011). By investigating the morphological and cellular defects characterizing these models, several laboratories have gained valuable insight into the unique role of SMN in motor neuron development and survival. The neuropathological signs that have been observed in these animal models and in human SMA patients are summarized in Figure 1. The deletion of the only copy of the *Smn* gene in mice leads to massive cell death prior to embryo implantation, highlighting the essential housekeeping function of SMN (Schränk et al., 1997). As SMA is characterized by reduced levels of full length SMN and not by complete ablation, a more suitable model for the human disease was engineered by introducing a human *SMN2* transgene into the *Smn* null background (*Smn*^{-/-}, *hSMN2*) (Hsieh-Li et al., 2000; Monani et al., 2000). These animals are born alive but develop a severe muscle paralysis and die shortly after birth or within one week of age. In this severe SMA model, as well as in the milder mouse model that harbors an additional *SMNΔ7* transgene (Monani et al., 2000), skeletal muscles show a progressive denervation starting at postnatal day 1. Interestingly, even at the disease end stage only about 20-35% of motor neurons are lost in the spinal cord (Cifuentes-Diaz et al., 2002; Monani et al., 2000), indicating that axonal

degeneration or otherwise compromised function of motor neurons is an early event in SMA, happening long before motor neuron cell death.

Despite the fact that muscle weakness and paralysis are the main phenotypes in SMA, generalized muscle denervation is not a prominent phenomenon (McGovern et al., 2008; Murray et al., 2008), although specific muscle groups, such as the ones in the head, neck, and trunk, have been shown to be more affected than other (Ling et al., 2011). The severe and progressive paralysis affecting SMA patients and SMA animal models can be explained in the light of the pronounced synaptic defects displayed by SMN-deficient motor neurons. First, work from the Mentis and Ko laboratories have shown that input from proprioceptive sensory neurons onto spinal motor neurons is significantly reduced in *SMN Δ 7* animals, due to a decrease in the number and size of glutamatergic synapses (Ling et al., 2010; Mentis et al., 2011). Second, remaining neuromuscular junctions (NMJs) are morphologically and functionally altered, showing phosphorylated neurofilament aggregation (Bowerman et al., 2011; Cifuentes-Diaz et al., 2002; Kariya et al., 2009; Kong et al., 2009; Ling et al., 2010; Ling et al., 2011; Murray et al., 2008), altered calcium homeostasis (Jablonka et al., 2007; Ruiz et al., 2010), and impaired neurotransmitter release (Kariya et al., 2008; Kong et al., 2009; Ling et al., 2011). Additionally, EM studies showed that presynaptic vesicles are unevenly distributed at the nerve terminals (Kariya et al., 2008; Kong et al., 2009). Interestingly, all of these phenotypes become apparent only after birth, while the embryonic development of the neuromuscular system is apparently normal (McGovern et al., 2008; Murray et al., 2010). After birth, NMJ maturation is significantly delayed in SMA animal models. Acetylcholine receptor (AChR) clustering fails to rearrange from an immature plaque shape to a mature perforated pretzel-like structure, and the expression of embryonic isotype AChR subunits is retained (Bowerman et al., 2011; Kariya et al., 2008; Kong et al., 2009). Taken together, these studies suggest that SMN function might be more relevant to NMJ maturation and maintenance than to motor neuron survival.

A role for SMN in motor neuron maturation is further supported by *in vitro* experiments on primary cultured motor neurons isolated from the severe SMA mouse model. These neurons show normal survival *in vitro* (Rossoll et al., 2003), but a striking reduction in the average length of the axonal process and in the area of the growth cone was observed. The importance of SMN for motor axons has been confirmed in other SMA animal models as well. Reduction of SMN levels via morpholino treatment in both *Xenopus* and zebrafish leads to severe defects in motor neuron axon outgrowth and pathfinding, and aberrant axon branching (McWhorter et al., 2003; Winkler et al., 2005; Ymlahi-Ouazzani et al., 2010). While a direct link between snRNP biogenesis and axon outgrowth in zebrafish has been suggested (Winkler et al., 2005), this finding remains controversial (Burghes and Beattie, 2009). Interestingly, work from the Beattie lab further suggested that in zebrafish the axonal defects caused by low levels of SMN are independent of SMN activity in snRNP assembly (Carrel et al., 2006). The expression of the SMN mutants Q282A or A111G, which retain the ability to perform snRNP assembly, failed to rescue the axonal phenotypes. On the other hand, when the C-terminal motif QNQKE - favoring SMN localization in axons (Zhang et al., 2007; Zhang et al., 2003) - was added to the snRNP assembly-incompetent *SMN Δ 7* protein (Zhang et al., 2007; Zhang et al., 2003), this resulted in normal motor neuron development. This finding could not be confirmed by complementation studies in mice with the A111G SMN mutant (Workman et al., 2009). Taken together, these results suggest that sufficient levels of SMN are necessary for motor axon development and maintenance, and imply that SMN function in motor neurons may go beyond snRNP biogenesis.

A first clue about the function of SMN in axons came from the observation of a significant reduction in the levels of β -actin mRNA and protein at the axon tip in motor neurons derived from the severe SMA mice (Rossoll et al., 2003). More recently, several labs have

shown that SMN deficiency caused defects in the cytoplasmic fate of several mRNAs (Akten et al., 2011; Fallini et al., 2011; Hubers et al., 2011), thus leading to the speculation that SMN may be necessary for the assembly of ribonucleoproteins responsible for the transport and local translation of axonal mRNAs (mRNPs). This hypothesis, which is further discussed in the following paragraphs, could explain the aforementioned defects in axon outgrowth and maintenance, as mRNA accumulation and local protein synthesis have been shown to be necessary for the regulation of axon outgrowth and growth cone dynamics (Jung et al., 2011; Satkauskas and Bagnard, 2007; Sotelo-Silveira et al., 2006).

SMN is an axonal protein

In all cell types, SMN localizes to both the cytoplasm and the nucleus, where it is found concentrated either in Cajal (coiled) bodies, or in closely associated nuclear structures termed gems, for gemini of Cajal bodies (Carvalho et al., 1999; Francis et al., 1998; Liu and Dreyfuss, 1996; Young et al., 2000). Early works with light and electron microscopy on motor neurons in the rat spinal cord identified SMN in association with polyribosomes in dendrites (Bechade et al., 1999) and with cytoskeletal filaments in axons (Pagliardini et al., 2000). Further studies on the human spinal cord demonstrated a progressive shift of SMN toward a prevalent axonal localization during axonogenesis and axonal sprouting (Giavazzi et al., 2006), supporting the idea of an axon-specific function for SMN.

Given the ability of SMN to shuttle from the nucleus to the cytoplasm, a role for SMN in the transport of mRNAs to the neuronal projections was hypothesized (Jablonka et al., 2000). This was further supported by *in vitro* studies that observed the presence of SMN in dendrites and at axonal branch points of cultured primary motor neurons (Jablonka et al., 2001), and in growth cone- and filopodia-like structures in neurites of differentiated P19 cells (Fan and Simard, 2002). A shorter splicing isoform of SMN called axonal SMN (a-SMN) comprising only the amino terminus has been reported to be mainly localized to axons and to have a neuritogenic effect in NSC34 cells (Setola et al., 2007). So far, a role of a-SMN in SMA has not been demonstrated.

An important step forward in support of a role for SMN in axonal mRNA regulation was the observation of rapid, bidirectional and cytoskeletal-dependent movements of fluorescently tagged SMN granules in axons of primary neurons by live cell imaging (Zhang et al., 2003). Depolymerization of microtubules impaired the long-range transport of SMN granules, whereas disruption of F-actin impaired short-range trafficking in axons (Zhang et al., 2003). Further live cell imaging studies in axons of primary motor neurons showed that SMN is transported both retro- and anterogradely at an average speed of 2 $\mu\text{m}/\text{sec}$ over long distances (Fallini et al., 2010), which is consistent with motor protein-dependent fast axonal transport. Importantly, essential snRNP components like Sm proteins are absent from these mobile axonal SMN granules and remain mainly restricted to the nucleus and cell body. Endogenous Sm proteins (Zhang et al., 2006) and epitope tagged SmD1 (Fallini et al., 2011) exhibited almost no colocalization with SMN granules in axons of primary motor neurons. Similarly, SMN granules in neurites of a human neuroblastoma cell line did not show colocalization with Sm proteins, as well as fibrillarin and EWS (Todd et al., 2010a). Based on these findings it appears unlikely that the axonal pool of SMN contributes to snRNP assembly. On the other hand, several studies have found that SMN colocalizes with core components of the SMN complex in neurites of neuronal cell lines as well as stem cell-derived and primary neurons. In an early study, SMN appeared to poorly colocalize with Gemin2 in axons of primary motor neurons (Jablonka et al., 2001). However, using high-resolution immunofluorescence, digital imaging analysis, and 3D reconstruction of growth cones in hippocampal neurons, it was later shown that 40-48% of SMN granules contained Gemin2 and Gemin3 (Zhang et al., 2006). Similar co-localization was observed in cortical

neurons, stem cell-derived motor neurons, and primary motor neurons (Zhang et al., 2006). Double label immunofluorescence studies have expanded this observation to the other Gemin proteins and Unrip (Fallini et al., 2011; Sharma et al., 2005; Todd et al., 2010b). Beyond co-localization, FRET analysis of fluorescent protein-tagged SMN, Gemin2, and Gemin3 proteins demonstrated interactions within individual granules (Zhang et al., 2006). Both SMN and Gemin proteins showed bidirectional movement in neurites of neuroblastoma cells (Todd et al., 2010b) and primary cultured neurons (Zhang et al., 2006). This has also been confirmed using a bimolecular fluorescence complementation (BiFC) approach, which showed that SMN dimerizes and interacts with Gemin2 not only in nuclear foci but also in actively transported axonal granules in primary motor neurons (Fallini et al., 2011). The function of this complex in axons is not clear yet, but taken together these data suggest that at least some components of the classical SMN complex may play a role in the noncanonical axonal function(s) of SMN (Cauchi, 2010). Indeed, several members of the SMN core complex have been shown to be involved in other processes of RNA post-transcriptional regulation that are independent of snRNP assembly, such as translational repression (Mourelatos et al., 2002; Murashov et al., 2007; Nelson et al., 2004) and modulation of cap-dependent and IRES-dependent translation (Fierro-Monti et al., 2006; Hunt et al., 1999; Pacheco et al., 2009). A role for Unrip in the assembly of mRNA transport complexes has also been suggested (Tretyakova et al., 2005). It still remains an open question whether all of the members of the classical SMN-Gemin complex are present in these axonal granules, or whether SMN in association with specific Gemin proteins forms entirely different multiprotein complexes.

Evidence for the role of SMN in axons: SMN-interacting proteins

Outside the classic SMN core complex, SMN has been shown to either directly interact or associate with a remarkably large number of other proteins with diverse functions (Coady and Lorson, 2011; Rossoll and Bassell, 2009). SMN-associated proteins include viral and cellular transcription factors, regulators of apoptosis, and growth factors. In this review we will focus on well characterized members of two groups among the SMN-associated proteins: 1) proteins associated with cytoskeletal dynamics, and 2) mRNA-binding proteins that are known to regulate mRNA transport, stability, and local translation in neurons. Most of these interaction partners have been found independently by different labs and their association with SMN has been confirmed by various biochemical assays and co-localization in cell culture. Often an interdependence of protein levels or even complementation of the mutant phenotypes has been observed.

SMN and the regulation of the cytoskeleton

Cytoskeletal integrity is essential for the correct functioning of the synaptic terminal and, in the axon, to ensure effective transport of proteins, mRNAs, and organelles (Holzbaur and Scherer, 2011). Several observations suggest that cytoskeletal dynamics may be impaired in SMA, thus contributing to motor neuron degeneration. First, the microtubule-destabilizing protein stathmin was found aberrantly upregulated under conditions of SMN deficiency, leading to an impaired microtubule network in SMN-deficient cells and reduced microtubule densities in distal axons of severe SMA mice. Knockdown of stathmin, on the other hand, reduced the microtubule defects in SMN-deficient cells and promoted axon outgrowth in motor neurons isolated from the severe SMA mouse model (Wen et al., 2010). Second, several SMN-associated proteins have been implicated in regulating the actin cytoskeleton, which plays important roles in recycling and tethering of synaptic vesicles in nerve terminals and maintaining its structural integrity (Dillon and Goda, 2005). Initially, SMN was shown to interact and to co-localize in motor neurons with the small actin-binding proteins profilin I and II (Giesemann et al., 1999). Further evidence for an association of SMN with the actin cytoskeleton came from the observation that SMN can modulate actin polymerization *in*

vitro (Sharma et al., 2005) and that knock-down of SMN leads to inappropriate activation of the RhoA actin-remodeling pathway via interaction with profilin II (Bowerman et al., 2007). Furthermore, the Rho-kinase-downstream targets cofilin, myosin-light chain phosphatase, and profilin IIa are differentially phosphorylated, leading to a neuron-specific change in the F-/G-actin ratio in SMN-deficient cells and an SMA mouse model (Nolle et al., 2011). Together, these data suggest a link between disruption of actin cytoskeletal dynamics and SMA pathogenesis. This was also supported by the finding that pharmacological inhibition of Rho-kinase prolongs survival of an intermediate SMA mouse model (Bowerman et al., 2010). Furthermore, the SMN-associated actin bundling protein Plastin 3/T-plastin (PLS3) acts as a protective modifier of SMA in humans and SMA animal models (Bernal et al., 2011; Dimitriadi et al., 2010; Oprea et al., 2008; Stratigopoulos et al., 2010). PLS3 overexpression can rescue axon outgrowth defects associated with SMN deficiency in cultured motor neurons from the severe SMA mouse model and in zebrafish embryos after knockdown of SMN. Invertebrate PLS3 orthologs act as modifiers in *C. elegans* and *D. melanogaster* animal models of SMA, suggesting that the connection between SMN and plastin-associated pathways is conserved (Dimitriadi et al., 2010). Currently it is not clear whether there is a direct link between dysregulation of actin dynamics and SMN function, or whether stabilization of the cytoskeleton may ameliorate SMA pathogenesis by an unrelated mechanism. A decrease of PLS3 levels in an SMA mouse model was restored by genetic ablation of profilin II, but without rescuing the SMA phenotype (Bowerman et al., 2009). Future experiments should address the effects of early overexpression of PLS3 on SMA pathogenesis in mouse models.

SMN and the regulation of neuronal RNA-binding proteins

While SMN has been shown to have the capacity to bind RNA *in vitro* (Bertrand et al., 1999), it does not contain any known RNA binding domain and an indirect interaction with mRNA via mRNA-binding proteins (mRBPs) appears more likely. Indeed, SMN has been found to interact with a variety of mRBPs that are known to regulate mRNA transport, stability, and local translation in neurons. Several of these proteins, including hnRNP U, hnRNP Q, and FMRP, but also EWS, Nucleolin, and Unrip have previously been identified as components of KIF5-associated RNA transport granules (Kanai et al., 2004). Generally, RBPs shuttle between the cytoplasm and the nucleus in varying degrees and play a multifunctional role in regulating several aspects of RNA metabolism. These include mRNA splicing, editing, transport, stability, and translation (Goldie and Cairns, 2011; Liu-Yesucevitz et al., 2011). Table 1 summarizes important features of a selected group of mRBPs, known to associate and co-localize with SMN in neurites. Frequently, interactions between SMN and mRBPs are impaired by SMA patient-derived missense mutations or small deletions in the Tudor domain or the C-terminal YG-box (Fallini et al., 2011; Hubers et al., 2011; Piazzon et al., 2008; Rossoll et al., 2002). This implies that the function of SMN in mRNP biogenesis may be abrogated in SMA patients. SMN-associated mRBPs share a number of characteristics that are discussed in more detail below.

First, SMN-associated RBPs frequently contain arginine and glycine (RG) rich domains that are often methylated, as it has been demonstrated for the RG rich domains in spliceosomal Sm proteins (Paushkin et al., 2002). This post-transcriptional modification is supposed to enhance the interaction with the SMN Tudor domain. The best characterized examples are KSRP and HuD, which interact with the Tudor domain of SMN in a methylation-dependent fashion (Hubers et al., 2011; Tadesse et al., 2008). This observation has led to the hypothesis that SMN can act as a molecular chaperone for methylated proteins involved in mRNA post-transcriptional regulation (Tadesse et al., 2008).

Second, hnRNP Q, FMRP, KSRP, HuD, and TIA-R, play a role in regulating the turnover of mRNAs that contain adenine/uridine-rich elements (AREs) in their 3' untranslated regions

(Barreau et al., 2005; von Roretz et al., 2011). A few of these transcripts have been shown to be affected by SMN deficiency, as discussed below.

Third, SMN, TIA-R, hnRNP Q, FMRP, KSRP, HuD, and TDP-43 have been found associated with RNA stress granules, which are discrete cytoplasmic granules that assemble and silence mRNAs in response to environmental stress (Kedersha and Anderson, 2009; von Roretz et al., 2011). The localization of these RBPs to cytoplasmic stress granules suggests a role in regulating their target mRNAs in response to stress. This connection is further strengthened by the finding that knock-down of SMN in neuronal cell lines reduced stress granule formation and sensitized cells to stress treatment. This effect was partially reverted by overexpression of the stress granule marker G3BP to restore stress granule formation (Zou et al., 2011).

Fourth, FMRP and HuD, as well as Unrip, have been shown to interact with nuclear mRNA export factors of the NXF protein family (Lai et al., 2006; Saito et al., 2004; Tretyakova et al., 2005). This interaction may serve to facilitate the nuclear export of their mRNA cargoes and their transport into neurites through interactions with motor proteins (Takano et al., 2007).

Finally, SMN-associated mRBPs often show reduced protein levels under conditions of SMN deficiency. Specifically, this has been demonstrated for hnRNP Q (Helmken et al., 2003) and KSRP (Tadesse et al., 2008), and a specific reduction in axonal protein levels has been found for HuD (Fallini et al., 2011). Taken together, these studies indicate a potential role for SMN in the regulation of mRNAs in the cytoplasm, perhaps by acting as an assembly factor for mRNPs analogous to its role in snRNP assembly (Fallini et al., 2011; Rossoll and Bassell, 2009).

Evidence for a role of SMN in axons: SMN and the regulation of cytoplasmic mRNA fate

Several observations in SMA animal and cellular models, including the specific defects at the NMJ and the association of SMN with various mRNA-binding proteins, have led to the hypothesis that SMN may facilitate the localization to the NMJ of an array of mRNAs that are necessary for its maturation and maintenance. The specific enrichment of the axon and growth cone with a subset of mRNAs has been shown to be essential for axon development, maintenance, and regeneration (Jung et al., 2011; Satkauskas and Bagnard, 2007; Sotelo-Silveira et al., 2006). Interestingly, no axon sprouting, even at a late stage of the disease, has been reported in the severe SMA animal models (Cifuentes-Diaz et al., 2002; Murray et al., 2008). Recently, we have shown that SMN deficiency in primary motor neurons leads to a dramatic reduction of poly(A) mRNA levels specifically in the axonal compartment (Fallini et al., 2011). This and the observed mislocalization or downregulation of mRBPs in motor axons, supports the hypothesis of SMN as a regulator of mRNP assembly. However, what mRNAs may be affected by low SMN levels, and what mechanisms are involved, is still not completely understood.

Reduced levels in motor neuron axons and growth cones of two important mRNAs, *β-actin* and candidate plasticity-related gene 15 (cpg15)/neuritin (Akten et al., 2011; Rossoll et al., 2003), can at least in part explain the defects in axonal sprouting and NMJ maintenance observed in SMA. *β-actin* is one of the major structural components of the highly dynamic growth cone and synapse. In SMA motor neurons, defects in the abundance of both *β-actin* mRNA and *β-actin* protein in growth cones have been described, accompanied by shorter axons and smaller growth cones (Rossoll et al., 2003). Interestingly, restoration of *β-actin* mRNA and protein levels in SMA motor neurons by cAMP treatment or via silencing of the

protein synthesis inhibitor PTEN was able to rescue the observed morphological axonal defects *in vitro* and improve survival of motor neurons in SMA mice (Jablonka et al., 2007; Ning et al., 2010). The exact mechanisms underlying these defects (i.e. mRNA transport, stability, or local translation), and how SMN is involved in these processes, is not known yet. However, SMN has been shown to interact with the β -actin mRNA binding proteins hnRNP R (Glinka et al., 2010; Rossoll et al., 2003), KSRP (Gu et al., 2002; Tadesse et al., 2008), and HuD (Atlas et al., 2004), supporting the idea that SMN may favor their binding to and the transport of β -actin mRNA to the growth cone. Recombinant hnRNP R was shown to directly interact with the 3'UTR of β -actin mRNA and knockdown of hnRNP R in zebrafish embryos and in cultured mouse motor neurons leads to reduced axon length and lower β -actin mRNA levels in axonal growth cones (Glinka et al., 2010), similar to what observed in SMA motor neurons (Rossoll et al., 2003). It remains to be seen whether other β -actin mRNA binding proteins such as ZBP1/IMP1-3 (Zhang et al., 2001) associate with SMN-containing complexes as well, and whether SMN deficiency affects their interaction with their target mRNAs.

Another important mRNA whose levels have been shown to depend upon SMN, is *cpg15*. *Cpg15* encodes a small, highly conserved GPI-anchored protein involved in neurite outgrowth (Fujino et al., 2008), axon regeneration (Karamoysoyli et al., 2008), and neuromuscular synaptogenesis and axon branching (Javaherian and Cline, 2005). Interestingly, *cpg15* mRNA is targeted by HuD, which binds a regulatory element (i.e. AU-rich element, ARE) in its 3'UTR and stabilizes the transcript (Wang et al., 2011). *Cpg15* mRNA also colocalizes with HuD and SMN in motor neuron axons (Akten et al., 2011). Furthermore, similar to what was observed for β -actin mRNA, *cpg15* transcript levels are significantly reduced upon SMN knockdown, and *cpg15* overexpression partially rescued axonal defects caused by SMN knockdown in zebrafish embryos (Akten et al., 2011).

The cyclin-dependent kinase inhibitor *p21^{cip1/waf1}* mRNA is another ARE-containing transcript that has been shown to be misregulated in SMA (Olaso et al., 2006; Tadesse et al., 2008; Wu et al., 2011). Interestingly, *p21^{cip1/waf1}* mRNA is bound by HuD and KSRP, which regulate its stability in opposite directions (Fujiwara et al., 2006; Hubers et al., 2011; Joseph et al., 1998; Tadesse et al., 2008). However, the role of *p21^{cip1/waf1}* in post-mitotic motor neurons is not clear yet, and the relevance of *p21^{cip1/waf1}* misregulation in SMA still needs to be elucidated. Since many of the mRBPs that have been shown to associate with SMN are ARE-binding proteins (see Table 1) (Barreau et al., 2005; Bolognani and Perrone-Bizzozero, 2008; von Roretz et al., 2011), it will be interesting to see whether other ARE-containing mRNAs are also affected by SMN deficiency. SMN may facilitate the interaction of ARE-binding proteins e.g. HuD onto ARE sequences of multiple localized mRNAs.

Although appealing, the hypothesis that the deficiency of one specific mRNA at the NMJ may be responsible for the neuronal degeneration observed in SMA is very unlikely. Indeed, mice in which the gene encoding β -actin (*Actb*) has been knocked out in motor neurons only, did not display defective NMJ maintenance or axon regeneration (Cheever et al., 2011). Although it is possible that the upregulation of alternative actin isoforms could compensate for the loss of β -actin expression in these animals, these results suggest that the defective localization of not one but a wide array of mRNAs at both the pre- and post-synaptic compartment of motor neurons and motor neuron afferent fibers contributes to the degeneration that characterizes SMA. Defective localization of a large number of transcripts in SMA is also supported by the finding that general poly(A) mRNAs levels are reduced in axons of SMN-deficient cultured motor neurons (Fallini et al., 2011). The identification of the mRNAs deregulated in SMA motor neurons will be important for our understanding of the pathogenic mechanisms that lead to motor neuron degeneration in SMA and possibly other neurodegenerative diseases.

Conclusions and future perspectives

In this review we have summarized the neuroanatomical basis and experimental evidence for the role of mRNA cytoplasmic dysregulation in SMA. The classical function of SMN in snRNP assembly and nuclear pre-mRNA splicing has been the main focus of SMA research since its discovery. However, despite the excellent work from several laboratories and the extraordinary progress in the understanding of the molecular steps involved in snRNP biogenesis, a direct link between splicing and SMA has not been shown. On the other hand, the new emerging evidence for non-canonical functions of SMN in motor axons may help explain the motor neuron-specific and mainly axonal pathology observed in SMA patients and animal models. However, these two theories are not mutually exclusive. The observation that most SMN-associated mRBPs contain methylated arginine residues and interact with SMN via its Tudor domain, as it has been shown for Sm proteins, suggests the possibility that the SMN complex may play a dual function in neurons, by favoring the assembly not only of Sm proteins and snRNAs, but also of mRBPs and their target transcripts (Figure 2). The defects in spliceosomal assembly and function observed in SMA animals may be then accompanied by a defective activity of SMN in the assembly of mRNPs, thus acting in concert to cause a severe alteration of mRNA metabolism.

Motor neurons are among the largest cells in the human body, with thin and extremely long axons connecting the central nervous system with the periphery. These highly specialized cells receive a number of different afferent fibers, including upper motor neurons, interneurons, and sensory neurons, and function as relays for central signals to the skeletal muscles. As each myelinated motor axon reaches its target, it divides into 20-100 unmyelinated terminal fibers, each of which innervates a single muscle fiber. These synaptic terminals are structurally complex, highly dynamic and respond to target derived growth factors and other cues. mRNA localization and local protein synthesis are processes that are required for synaptic development, function and activity (Lisiecka et al., 2009; Swanger and Bassell, 2011; Wang et al., 2010; Wiersma-Meems et al., 2005), as is also underlined by the involvement of other mRBPs in neurologic diseases, such as FMRP in fragile-X syndrome (Bassell and Warren, 2008). Thus, it is possible that deficiencies in cytoplasmic mRNA regulation may be responsible for the defects observed at both pre- and post-synaptic compartments in SMA motor neurons. Indeed, genetic ablation of the protein synthesis inhibitor PTEN in SMA mice rescued motor neuron cell death, probably by restoring the levels of β -actin and other proteins at the growth cones or presynaptic terminals of SMA motor neurons. This suggests that upregulation of signaling pathways for mRNA trafficking and local protein synthesis in motor neurons could represent a novel target for therapy in SMA (Ning et al., 2010).

Although a substantial body of evidence supports a role for SMN in cytoplasmic mRNA regulation, the exact molecular mechanisms and their relevance for SMA still need to be determined and many open questions remain. What is the molecular composition of SMN-containing axonal granules? What other mRBPs are associated with SMN and, importantly, what mRNAs are mislocalized or misregulated upon SMN depletion? Do these defects contribute to the SMA pathology? Furthermore, it will be important to dissect the molecular mechanisms at the basis of these defects. Is SMN important for mRNP assembly, for their axonal and dendritic transport, or for the local translation of mRNAs at synapses? Understanding the role of SMN in mRNA metabolism in the cell bodies, axons, and dendrites of motor neurons will be important not only to elucidate the molecular mechanisms at the basis of motor neuron death in SMA and possibly other motor neuron diseases, but also to rationally design therapeutic approaches to delay or even prevent motor neuron loss in this devastating disease.

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Figure 1. Neuropathological hallmarks of SMA

Schematic representation of anatomical findings linked to reduced SMN levels in human SMA patients and in SMA animals. Spinal motor neurons located in the anterior horn of the spinal cord show increased cell death, defective innervation of the target skeletal muscle, and reduction in the number of afferent synapses with proprioceptive neurons. Also, heterotopic motor neurons located around the gray matter of the anterior horn have been reported in humans (Simic et al., 2008). Neuromuscular junctions (NMJs) in SMA are characterized by neurofilament (NF) aggregates, reduced neurotransmitter (NT) release, a delayed switch to adult acetylcholine receptor (AChR) subunits, and signs of denervation.

Figure 2. Molecular functions of SMN

Schematic representation of the proposed functions for SMN in neurons. SMN, present as part of the classical SMN complex, plays an essential role in small nuclear ribonucleoprotein (snRNP) biogenesis. An additional function in the assembly of mRNA-containing RNPs has been proposed, whereby SMN facilitates the recognition of RNA-binding proteins with their targets, and their transport along motor neuron axons.

Table 1

SMN-associated mRNA-binding proteins

Protein	RNA-binding domain	Selected target mRNAs	mRNA motifs	Methylation	Suggested roles in RNA regulation	Stress granule component	References
hnRNP R	3 RRM, RGG box	<i>β-actin</i> , <i>AANAT</i>	<i>unknown</i>	PRMT1	pre-mRNA splicing, mRNA stability, mRNA transport	<i>unknown</i>	Rossoll et al., 2002
hnRNP Q (<i>a.k.a.</i> <i>GRYBP</i> , <i>SYNCRIP</i> , and <i>NSAP1</i>)	3 RRM, RGG box	<i>IP3R1</i> , <i>AANAT</i> , <i>c-Fos</i>	ARE	PRMT1	pre-mRNA splicing, mRNA editing, mRNA stability, mRNA transport, translation	Yes	Mourdelatos et al., 2001; Rossoll et al., 2002
hnRNP U	RGG box	<i>Cox2</i> , <i>TNFα</i> , <i>Slh</i>	ARE	PRMT1	RNA transcription, mRNA stability	<i>unknown</i>	Liu and Dreyfuss, 1996
FMRP	2 KH, RGG box	<i>P50D95</i> , <i>MAP1B</i> , <i>p110b</i> , <i>K^v4.2</i>	ARE, G quartet, FMRP kissing complex	PRMT1	mRNA stability, translation inhibition	Yes	Piazzon et al., 2008
FBP1	4 KH	<i>nucleophosmin</i> , <i>Cox2</i>	<i>unknown</i>	<i>unknown</i>	mRNA stability, translation inhibition	Yes	Williams et al., 2000
KSRP (<i>a.k.a.</i> <i>FBP2</i> , <i>ZBP2</i> , and <i>MARTA1</i>)	4 KH	<i>β-actin</i> , <i>p21</i> , <i>Stathmin</i>	ARE	CARM1	mRNA stability, translation inhibition	Yes	Tadesse et al., 2008
HuD	3 RRM	<i>β-actin</i> , <i>cpg15/neuritin</i> , <i>p21</i> , <i>GAP43</i> , <i>Tau</i> , <i>Homer</i> , <i>ACHE</i>	ARE	CARM1	mRNA stability, transport, translation	Yes	Akten et al., 2011; Fallini et al., 2011; Hubers et al., 2011
TDP-43	2 RRM	<i>HDAC6</i> , <i>NF-L</i> , <i>MAP1B</i>	GU _n	<i>unknown</i>	pre-mRNA splicing, mRNA stability	Yes	Wang et al., 2002
TIAR	3 RRM	<i>eIF4A</i> , <i>eIF4E</i> , <i>eEF1B</i> , <i>c-Myc</i> , <i>β(2)-adrenergic receptor</i>	ARE	<i>unknown</i>	pre-mRNA splicing, translation	Yes	Hua and Zhou, 2004