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Spinal Muscular Atrophy and a Model for Survival of Motor Neuron Protein Function in Axonal Ribonucleoprotein Complexes

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Abstract

Spinal muscular atrophy (SMA) is a neurodegenerative disease that results from loss of function of the *SMN1* gene, encoding the ubiquitously expressed survival of motor neuron (SMN) protein, a protein best known for its housekeeping role in the SMN–Gemin multiprotein complex involved in spliceosomal small nuclear ribonucleoprotein (snRNP) assembly. However, numerous studies reveal that SMN has many interaction partners, including mRNA binding proteins and actin regulators, suggesting its diverse role as a molecular chaperone involved in mRNA metabolism. This review focuses on studies suggesting an important role of SMN in regulating the assembly, localization, or stability of axonal messenger ribonucleoprotein (mRNP) complexes. Various animal models for SMA are discussed, and phenotypes described that indicate a predominant function for SMN in neuronal development and synapse formation. These models have begun to be used to test different therapeutic strategies that have the potential to restore SMN function. Further work to elucidate SMN mechanisms within motor neurons and other cell types involved in neuromuscular circuitry hold promise for the potential treatment of SMA.

1 Spinal Muscular Atrophy and Survival of Motor Neuron Protein

Spinal muscular atrophy (SMA) is an inherited autosomal recessive neurodegenerative disease, primarily affecting α-motor neurons of the lower spinal cord, in which proximal muscles are more severely affected then the distal muscles. SMA has an estimated incidence between 1-in-6,000 and 1-in-10,000 births, and about 1 in 35–40 people are genetic carriers (Wirth et al. 2006). SMA is classified clinically by age of onset and highest level of motor function achieved (Wirth et al. 2006; Lunn and Wang 2008; Oskoui and Kaufmann 2008). In its most severe form, known as SMA type I or Werdnig-Hoffman Disease (Online *Mendelian Inheritance in Man* (OMIM) #253300), SMA is the most common genetic cause of infant mortality and the second most common lethal, autosomal recessive disease after cystic fibrosis (Melki et al. 1994). Degeneration and death of the anterior horn motor neurons in the brain stem and spinal cord produce weakness in the limb muscles, as well as in muscles involved in swallowing and breathing (Nicole et al. 2002). Children with SMA present with generalized muscle weakness and hypotonia, and either do not acquire or progressively lose the ability to walk, stand, sit, and, eventually, move. Previously, SMA type I patients were predicted to die before the age of two, but in recent years, more proactive clinical care has improved survival (Oskoui et al. 2007; Oskoui and Kaufmann 2008). Intermediate SMA type II (OMIM #253550) is characterized by the onset after the

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age of 6 months. Patients acquire the ability to sit, but can never walk unaided. Patients with juvenile SMA type III (Kugelberg Welander disease; OMIM #253400) typically reach major milestones and can walk independently. The adult form, SMA type IV (OMIM #271150), is characterized by an age of onset beyond 30 years and relatively mild motor impairment.

SMA is caused by deletions or mutations of the survival of motor neuron gene (*SMN1*), which was originally cloned and characterized by Melki and colleagues (Lefebvre et al. 1995). *SMN1* is an essential gene in divergent organisms, in which null mutations are lethal during early development (Schmid and DiDonato 2007). The survival of motor neuron (SMN) protein is ubiquitously expressed in all cells and tissues, with high levels in the nervous system, especially in spinal cord (Battaglia et al. 1997). Unlike other species that have only one copy of the *SMN* gene (e.g., mice), the *SMN* gene is present on human chromosome 5q13 as a single copy of the telomeric *SMN1* gene, and a variable number of centromeric *SMN2* genes. *SMN2* appears to be unique to humans, since chimpanzees contain multiple copies of *SMN1*, but no *SMN2* (Rochette et al. 2001). The majority of mRNAs (90%) from *SMN1* encode for the full-length protein; whereas, the majority of mRNAs (90%) from *SMN2* encode for a truncated and unstable protein lacking the carboxy-terminal exon-7 due to a translationally silent mutation in an exonic splicing enhancer (Lorson et al. 1999). The full-length transcripts of *SMN1* and *SMN2* encode proteins with an identical sequence. The most commonly inherited forms of SMA are caused by large deletions that inactivate the *SMN1* gene and, while the unique presence of *SMN2* in humans can protect against lethality, a neurodegenerative process occurs, leading to SMA. A major challenge is to understand how a reduction in total SMN levels results in neuronal dysfunction that leads to SMA.

The function of SMN was addressed by Dreyfuss and colleagues, who were seeking to identify proteins binding to hnRNP-U, a member of a family of heterogenous ribonucleoproteins. SMN was identified from their screen and shown to localize to nuclear structures, termed gems, based on their proximity to coiled bodies/Cajal bodies (i.e., Gemini of Cajal bodies) (Liu and Dreyfuss 1996). SMN was found tightly associated with a novel protein, SIP1, subsequently referred to as Gemin 2, and together, they form a specific complex with several spliceosomal snRNP proteins (Liu et al. 1997). The Dreyfuss lab and others subsequently identified other SMN interacting proteins (see Sect. 2.2), and extensively studied the critical role of the SMN–Gemin multiprotein complex in the assembly of spliceosomal snRNP s (Gubitz et al. 2004; Yong et al. 2004; Battle et al. 2006a; Eggert et al. 2006). The SMN–Gemin complex acts as a specificity factor to promote high-fidelity interactions between Sm core proteins and snRNAs, which prevent promiscuous interactions with other RNAs (Pellizzoni et al. 2002b). Recent data indicate that SMN deficiency alters stoichiometry of snRNAs and leads to splicing defects for numerous genes in all cells, including motor neurons (Zhang et al. 2008). In SMN-deficient mouse models of SMA, there is a preferential reduction in snRNP species that function in the minor spliceosome required for processing a rare class of introns (Gabanella et al. 2007). The function of the SMN–Gemin complex in snRNP assembly thus represents the most well understood function of SMN.

A major question needed to be addressed is how reduction of SMN in all tissues leads to a preferential impairment of motor neurons, although other neurons (e.g., sensory) and other tissues (e.g., muscle fibers) are also affected. Since the effects of SMN-deficiency on snRNP species and the stoichiometry of spliced introns appear to vary across tissues, one can also speculate that motor neurons may be more vulnerable to splicing alterations; however, to test this model, it would be necessary to demonstrate that altered splicing can have effects on protein expression and sequence composition that are deleterious to motor neurons. It is more than likely, however, that there are other functions of the SMN–Gemin complex, or

even other types of SMN complexes, which contribute to the pathogenic mechanism in motor neurons. Inasmuch as SMN is localized to both the nucleus and cytoplasm (Young et al. 2000a), it becomes critical to understand whether the cytoplasmic pool of SMN is involved in activities other than that involved in the assembly of snRNPs.

2 SMN Domains and Interacting Proteins

2.1 SMN Domain Structure

The *SMN1* gene contains nine exons and eight introns in a genomic region of ca. 20 kb. The ubiquitously expressed *SMN1* transcript of ca. 1.7 kb encodes a highly conserved 294 amino acid protein of 38 kDa. SMN contains several functionally important regions for self assembly and protein interaction (see Fig. 1). The N-terminal part contains the binding sites that have been implicated in the interaction with Gemin2 from amino acids 13–44 (Liu et al. 1997) and 52–91 (Young et al. 2000b). The region encoded by exon2 has been shown to bind RNA directly in vitro (Lorson and Androphy 1998; Bertrandy et al. 1999). Exon 3 encodes a central Tudor domain, comprising amino acids 90–160. Tudor domains are conserved sequence motifs that were originally described for the *Drosophila* tudor protein. They are thought to mediate protein–protein interactions and are often found in RNA-associated proteins (Ponting 1997; Selenko et al. 2001). Tudor domain proteins have been shown to interact with proteins that contain methylated arginine and lysine residues (Brahms et al. 2001; Sprangers et al. 2003; Cote and Richard 2005). The SMN Tudor domain mediates interaction with arginine and glycine-rich motifs in several proteins (Meister et al. 2002; Paushkin et al. 2002; Gubitz et al. 2004), including the Sm core proteins. Symmetrical dimethylation of specific arginine residues enhances their affinity for SMN (Friesen et al. 2001a, b; Boisvert et al. 2002; Hebert et al. 2002; Meister and Fischer 2002). Exons 4–6 contain three stretches of poly-proline sequences that mediate the interaction with the small actin-binding protein profilin (Giesemann et al. 1999b). A conserved tyrosine/glycine-rich motif (YG box) that is found in many RNA-binding proteins is localized in exon 6, from amino acid 258–279. Regions required for dimerization or oligomerization of SMN have been mapped to self-association domains in exon 2b (52–91) and exon 6 (242–279) (Lorson et al. 1998; Young et al. 2000b).

SMA is characterized by a reduced level of full length SMN in the presence of SMN that lacks exon 7 (SMNΔ7). Although the expression of the SMNΔ7 isoform is beneficial, it cannot fully compensate for loss of full length SMN (Le et al. 2005). Therefore, the function of the protein domain encoded by exon 7 from amino acid 280–294 is of special interest. SMNΔ7 has been reported to encode an unstable and rapidly degraded protein (Le et al. 2000; Lorson and Androphy 2000) that is deficient in oligomerization activity (Lorson et al. 1999; Young et al. 2000b), binding to Sm core proteins (Pellizzoni et al. 1999), and formation of gems (Frugier et al. 2000). The SMNΔ7 protein has a twofold shorter half-life than full length SMN in cells, despite similar turnover rates, mediated by the ubiquitin-proteasome system in an in vitro assay (Burnett et al. 2008). SMNΔ7 can be stabilized by the coexpression of full length SMN, suggesting that it is stabilized by recruitment into oligomeric SMN complexes (Le et al. 2005). It is noteworthy that exon7 has also been shown to contain a cytoplasmic targeting signal that is required for the active transport of SMN into neuronal processes (Zhang et al. 2003).

Although in the large majority of cases SMA is caused by homozygous deletion of the SMN1 gene, a small percentage of SMA patients bear one *SMN1* copy with small mutations. This is of considerable interest, since missense mutations may affect certain properties of SMN without causing a general loss of function. As depicted in Fig. 1, most SMA mutations have been found clustered in the tudor domain (W92S, V94G, G95R, A111G, I116F, Y130C, E134K, and Q136E) and in exon 6, within and near the Y/G box

(L260S, S262G, S262I, M263R, M263T, S266P, Y272C, H273R, T274I, G275S, G279C, and G279V). This distribution of mutations suggests that the Tudor domain and the Y/G box with its flanking region are essential for SMN function. Studying these and synthetic SMN mutations may make it possible to uncouple functions required for snRNP assembly and proposed axonal functions in genetic rescue experiments (Beattie et al. 2007).

2.2 SMN Interacting Proteins

SMN is part of a well-characterized complex that facilitates assembly of Sm proteins on multiple U snRNAs to form the snRNP core (Meister et al. 2002; Paushkin et al. 2002; Gubitz et al. 2004; Kolb et al. 2007). To date, nine proteins have been identified as core components of this complex: SMN, Gemins 2–8 and unrip. SIP1 (Smn interacting protein) (Liu and Dreyfuss 1996), subsequently referred to as Gemin2, was identified as the first component of the SMN complex and shown to have a critical function in the assembly of spliceosomal snRNP s (Fischer et al. 1997; Liu et al. 1997), which has since been extensively studied by the Dreyfuss lab and others (Gubitz et al. 2004; Yong et al. 2004; Battle et al. 2006a; Eggert et al. 2006). Gemin2, Gemin3, and Gemin8 bind SMN directly (Otter et al. 2007). Gemin3 is a DEAD box RNA helicase (Charroux et al. 1999) and Gemin5 is the snRNA binding protein (Gubitz et al. 2002; Battle et al. 2006b). Gemin8 is needed for the structural organization of the SMN–Gemin complex (Carissimi et al. 2006). This SMN–Gemin core complex is found associated with spliceosomal Sm/LSm core proteins (SmB/B′, D1–3, E, F, G, LSm10, 11). It catalyzes the assembly of ring structures consisting of seven Sm core proteins onto stem loop structures of uridine-rich snRNAs (Chari et al. 2008). The high molecular weight of the SMN complex suggests that SMN and other core components are present as multimers. Oligomerization of SMN is also a prerequisite for high-affinity binding of the SMN complex to spliceosomal snRNPs (Pellizzoni et al. 1999).

In addition to the SMN complex components, SMN interacts directly or is associated with a remarkably large number of other proteins (see Table 1). This suggests that SMN may have a pleiotropic function that goes beyond the well-characterized role in snRNP assembly.

The largest group of SMN-associated proteins comprises components of RNP complexes that play a role in some aspects of RNA metabolism. Many of them contain domains that are enriched in arginine and glycine residues that are required for interaction with SMN (Meister et al. 2002; Paushkin et al. 2002). Arginine residues in these RG-rich domain are often methylated, which can enhance the interaction with SMN (Paushkin et al. 2002). The other interaction partners are very heterogenous and include viral and cellular transcription factors, regulators of apoptosis, and growth factors.

2.3 SMN Interacting Proteins Associated with Actin Metabolism

Interestingly, several interacting proteins have been implicated in either β-actin mRNA transport or in the regulation of actin dynamics. Examples of interacting partners that could potentially affect actin-based functions are briefly outlined as follows and further discussed below.

1. SMN interactions with regulators of actin dynamics. SMN was found to interact with the small actin-binding proteins profilin I and II in yeast-two-hybrid assays and to colocalize in motor neurons (Giesemann et al. 1999a). Profilin II and SMN were shown to colocalize in neurites and in growth cones of differentiating rat PC12 cells (Sharma et al. 2005). Antisense knockdown of profilin I and II isoforms inhibited neurite outgrowth of PC12 cells and caused accumulation of SMN and its associated proteins in cytoplasmic aggregates. SMN can modulate actin polymerization in vitro by reducing the inhibitory effect of profilin IIa. Therefore,

reduced SMN-levels in SMA could disturb the normal regulation of microfilament growth by profilins and cause defects in axonal outgrowth. In a related study, SMN knockdown in PC12 cells altered the expression pattern of profilin II, leading to an increased formation of ROCK/profilin IIa complexes (Bowerman et al. 2007). Inappropriate activation of the RhoA/ROCK actin-remodeling pathway may result in altered cytoskeletal integrity and impaired neurite outgrowth.

2. Reduced actin expression in Smn-deficient distal axons. The distal distribution of β-actin mRNA and protein is defective in SMN-deficient motor neurons (see Sect. 3.1) (Rossoll et al. 2003). Treatment of primary motor neurons from *Smn*−/−; *SMN2* embryos with a cell-permeable cAMP analog (8-CPT-cAMP) not only leads to an increased β-actin mRNA and protein level in the growth cone but also normalized the axon length and growth cone size defects to control levels (Jablonka et al. 2007). Thus, pharmacological intervention can increase distal β-actin mRNA and protein levels, and at the same time correct morphological axonal defects observed in SMA.

3. *SMN and transport/regulation of* β*-actin mRNA and other transcripts.* SMN interacts with several proteins thought to be involved in the transport and post-transcriptional regulation of β-actin mRNA and other transcripts: KSRP/ZBP2/MARTA1 (Tadesse et al. 2008), FBP (Williams et al. 2000b), hnRNP R, and hnRNP Q (Mourelatos et al. 2001; Rossoll et al. 2002, 2003). This is discussed in more detail in Sects. 3.1 and 3.2.

4. *Protection from SMA by plastin 3 expression.* Plastin 3/T-plastin (PLS3) has been identified as a protective modifier shown to physically interact with SMN complexes in a study of siblings discordant for SMA (Oprea et al. 2008). SMA-unaffected *SMN1*-deleted females exhibit significantly higher expression of PLS3 than their SMA-affected siblings. The actin-bundling protein PLS3 is important for axonogenesis through the increase in the F-actin level. Proteins of the fimbrin/plastin family share the unique property of cross-linking actin filaments into tight bundles that assist in stabilizing and rearranging the organization of the actin cytoskeleton in response to external stimuli, as well as perhaps by actin filament stabilization and anti-depolymerization activities (Oprea et al. 2008). Overexpression of PLS3 in SMN-deficient motor neurons rescued the axon length and outgrowth defects associated with SMN down-regulation.

5. *SMN complexes with ZPR1 and eEF1A.* SMN assembles into complexes with the zinc finger protein ZPR1 and eukaryotic translation elongation factor 1A (eEF1A) (Mishra et al. 2007). eEF1A binds to actin and has a noncanonical function in actin bundling (Gross and Kinzy 2005).

All considered, these data emphasize a potential role of SMN in the regulation of the axonal actin cytoskeleton, in part, by influencing β-actin mRNA transport and local translation and/or actin filament formation. Failure of locally modulated β-actin synthesis or F-actin localization in axon terminals may have a severe impact on axon growth, synapse differentiation and maintenance, the scaffolding of regulatory molecules such as synapsin, and the trafficking and release of synaptic vesicles (Shupliakov et al. 2002).

3 Localization of SMN and Links to Axonal mRNA Regulation

Previous immunocytochemical studies have localized SMN in dendrites and axons of spinal cord motor neurons in vivo (Bechade et al. 1999; Pagliardini et al. 2000). These immuno-EM analyses also depicted SMN on cytoskeletal filaments and associated with polyribosomes. Several immunofluorescence (IF) studies have detected Smn in neurites of

cultured P19 cells (Fan and Simard 2002) and axons of cultured motor neurons (Rossoll et al. 2002). High-resolution imaging has revealed the presence of SMN in granules that localize to axons and growth cones of cultured neurons and align along microtubules (Zhang et al. 2003). As shown in Fig. 3, SMN-containing granules are not only abundant in the cell body and dendrites, but also along the axons and growth cones of motor neurons. SMN granules were shown by IF double labeling and fluorescence in situ hybridization (FISH) to colocalize with mRNA and ribosomes. Fluorescence imaging methods applied to living neurons showed that SMN granules are actively transported into neuronal processes and growth cones at rates over 1 $\mu m\ s^{-1}$ (Zhang et al. 2003), consistent with fast axonal transport. Depolymerization of microtubules impaired the long-range transport of SMN granules, whereas disruption of F-actin impaired short-range trafficking (Zhang et al. 2003).

Double label IF studies have suggested possible colocalization between SMN and Gemin proteins in neurites of PC12 cells (Sharma et al. 2005). However, significant levels of SMN appear not to colocalize with Gemin2 in axons of primary motor neurons (Jablonka et al. 2001). A later study, using high-resolution IF, digital imaging analysis, and 3D reconstruction of growth cones, showed that a population of SMN granules contained Gemin2 and Gemin3 (30–40%); however, the majority of SMN lacked Gemin proteins (Zhang et al. 2006). FRET analysis of fluorescently tagged SMN and Gemin proteins demonstrated interactions within individual granules, which were also observed to move as a complex in live neurons (Zhang et al. 2006). The QNQKE sequence from exon-7 was necessary for the sorting of the SMN–Gemin complex into the cytoplasm (Zhang et al. 2007). Of interest, the spliceosomal Sm proteins, necessary for snRNP assembly, were confined to the cell body and exhibited little colocalization with Smn–Gemin complex in neuronal processes (Zhang et al. 2006). Collectively, these studies indicate the presence of distinct SMN ribonucleoprotein complexes in neuronal processes that may play a role in mRNA regulation.

There appears to be some relationship between the neuritogenic effects of SMN and its cytoplasmic localization. The SMN Δ 7 form of SMN, which is the predominant form in SMA, was enriched within the nucleus, with much lower levels in the cytoplasm (Zhang et al. 2003). A QNQKE sequence with the carboxy-terminus of SMN was found to be necessary for cytoplasmic localization. Overexpression of SMN was neuritogenic in comparison to SMN Δ 7. This defect could be rescued by fusion of the membrane targeting sequence from GAP-43. SMN Δ 7 fused to this GAP-43 sequence now was targeted to axons and stimulated neurite growth.

A shorter splicing isoform of SMN called axonal SMN (a-Smn) has been reported to have a neuritogenic effect in NSC34 cells (Setola et al. 2007). The identified mRNA retains intron 3, which contains an in-frame stop codon. a-SMN is encoded by exons 1–3 and a small region of intron 3 that is not conserved between species. Future work is needed to address how prevalent this form of SMN is in axons. However, there has been some controversy as to whether this form of SMN could play a role in the pathogenesis of SMA (Burghes 2008). Most missense mutations found in SMA patients lie downstream of exon 3 and are clustered in exon 6. Also, there is strong evidence that SMA is caused by lack of exon7, and we know from experiments with SMA mouse models that expression of full-length murine Smn cDNA and human SMN2 can rescue the mutant phenotype. It will be interesting to assess the effects of a-SMN on rescue of axon phenotypes in animal models (discussed below).

3.1 Mechanism of β-Actin mRNA Localization in Neurons and Possible Connection to SMN and SMA

RNA localization is an essential and highly conserved biological mechanism for protein sorting that plays critical roles in neuronal polarity, axon guidance, and synaptic plasticity

(Kindler et al. 2005; Bramham and Wells 2007; Lin and Holt 2008). Several mRNAs have been shown to be targeted to dendrites and/or axons, both in vivo and in cultured neurons. The molecular mechanism of mRNA localization involves recognition of *cis*-acting sequences within the 3′-untranslated region (UTR) by specific mRNA binding proteins and accessory factors, which are assembled into large mRNP complexes, often termed "*granules*" (Kiebler and Bassell 2006). mRNA granules are actively transported along microtubules and contain mRNA binding proteins, translation factors, ribosomal subunits, and accessory factors. mRNAs present within transport granules are often translationally repressed, which become derepressed in response to receptor signaling (Besse and Ephrussi 2008).

β-actin mRNA represents the most extensively studied localized mRNA, and hence provides a logical starting point to assess a possible role for SMN in the mechanism of mRNA granule localization. FISH analysis revealed that β-actin mRNAs are localized to developing axons and growth cones of primary cortical neurons in culture (Bassell et al. 1998). EM analysis showed the presence of polyribosomes within axon growth cones. A neurotrophin (NT-3) signaling pathway increased localization of β-actin mRNA from the cell body into processes and growth cones, while γ-actin mRNA remained in the cell body (Bassell et al. 1998; Zhang et al. 1999). The neurotrophin-induced localization of β-actin mRNA resulted in a protein-synthesis dependent increase in β-actin protein levels in growth cones (Zhang et al. 2001). The molecular mechanism of β-actin mRNA localization in chick forebrain neurons was shown to involve a highly conserved zipcode (54 nt) sequence, only present in the 3′UTR of β-actin, which is recognized by the mRNA binding protein, zipcode binding protein ZBP1 (Ross et al. 1997). ZBP1 has four KH-domains, which are necessary for binding to the zipcode, RNA granule formation, and association with the cytoskeleton (Farina et al. 2001).

ZBP1 also has NLS and NES and has been shown to shuttle into the nucleus where it first associates with nascent β-actin mRNA (Oleynikov and Singer 2003). Once in the cytoplasm, ZBP1 is hypothesized to function as an adapter for microfilament- and/or microtubule-dependent motors to facilitate the transport of β-actin mRNA in fibroblasts and neurons, respectively. In neurons, antisense-mediated disruption of ZBP1 binding to the zipcode impaired NT-3-induced localization of β-actin mRNA into neurites, resulting in reduced enrichment of β-actin protein and impaired growth cone dynamics (Zhang et al. 2001). In *Xenopus* neurons, binding of ZBP1 (VgRBP) to the β-actin zipcode was required for local β-actin synthesis and axon guidance in vitro in response to BDNF or netrin (Leung et al. 2006; Yao et al. 2006). Recent evidence indicates that ZBP1 acts to repress β-actin mRNA translation, and that in response to Src activation, ZBP1 is phosphorylated, resulting in its release from the mRNA, allowing for translation (Huttelmaier et al. 2005). Since β-actin appears to be the preferred actin isoform to induce rearrangements of the actin cytoskeleton beneath the plasma membrane (Bassell et al. 1998), it is hypothesized that local β-actin mRNA translation is necessary to promote enrichment of β-actin protein at the membrane and influence growth cone motility and guidance. ZBP1 is thus a critical factor for both β-actin mRNA transport and local protein synthesis; however, there may be other proteins within the complex that contribute to these processes, and Smn is attractive candidate based on its trafficking in the form of RNA granules.

In a study providing the first link between Smn and β-actin mRNA, Smn-deficient motor neurons, cultured from a transgenic mouse model of SMA, were shown to have reduced localization of β-actin mRNA in axons and reduced levels of β-actin protein in axonal growth cones (Rossoll et al. 2003). Of interest, Smn-deficient motor neurons displayed only defects in axonal morphology, that is, shorter axons and smaller growth cones, whereas dendrites appeared otherwise normal in morphology. These results suggest some role of

SMN in the molecular mechanism of β-actin mRNA localization to axons. These results also imply that axonal defects in SMA may be due, in part, to altered localization of β-actin mRNA. Future work will be necessary to explore a possible interaction between SMN and ZBP1. However, it also possible that Smn may interact with ZBP2 to facilitate β-actin mRNA localization. ZBP2 is a KH-domain-containing protein that is predominantly localized to the nucleus, where it facilitates the hand-off of β-actin mRNA to ZBP1 that exports the mRNA into the cytoplasm (Gu et al. 2002; Pan et al. 2007). Of interest, SMN has been shown to interact with ZBP2 homologs, viz., FBP and KSRP. By yeast two-hybrid and co-immunoprecipitation (IP) analysis, SMN was shown to bind FUSE binding protein (FBP) (Williams et al. 2000a), a KH domain-containing RNA binding protein that regulates the stability of GAP-43 mRNA (Irwin et al. 1997), which is localized to axons and growth cones (Smith et al. 2004). More recent work has shown that SMN binds KSRP (Tadesse et al. 2008), a neuron-specific splicing factor that is also involved in targeting AU-rich mRNAs for degradation (Min et al. 1997). KSRP was shown to be arginine-methylated and interact with the Tudor domain of SMN. Smn and KSRP colocalized in granules within neurites and missense mutations in the Tudor domain that cause SMA abolished the colocalization with KSRP. In SMA transgenic mice, a KSRP target mRNA, p21 (cip/waf1), was increased in spinal cord, suggesting possible impairment of mRNA stability. How this relates to altered mRNA localization is unknown, but there are known links between mRNA localization and stability mechanisms; thus, it will be important for future work to assess a possible alternative or related role of SMN in regulation of mRNA stability in axons. Other studies have demonstrated a role for MARTA1, the rat KSRP ortholog, to bind the dendritic targeting element of MAP2 mRNA and facilitate its targeting in dendrites (Rehbein et al. 2002). Since Smn is localized to dendrites and axons (Bechade et al. 1999; Zhang et al. 2006), it will be important to assess a possible role of Smn in regulation of dendritic mRNA localization, which may affect postsynaptic function in spinal cord motor neurons.

3.2 SMN Interactions with hnRNP R and hnRNP Q: Another Possible Link to β-Actin mRNP Complexes

Yeast two-hybrid (Y2H) screens using SMN as a bait have identified two highly related proteins, hnRNP R (heterogenous nuclear ribonucleoprotein R) and hnRNP Q, previously discovered as glycine–arginine–tyrosine-rich RNA-binding protein (gry-rbp). hnRNP R and Q interact with wild-type SMN, but not with mutated versions of the protein found in SMA patients (Rossoll et al. 2002). In a parallel Y2H study, Mourelatos et al. have identified three human splicing isoforms of hnRNP Q as SMN interacting proteins (Mourelatos et al. 2001). hnRNP Q3/gry-rbp encodes a protein of 623 aa that is composed of an N-terminal acidic domain, followed by three consecutive RNA recognition motifs (RRM1–3) and an arginine- and glycine-rich domain (RGG box). hnRNP Q3 and hnRNP R share 82% identity and 90% similarity on the protein level. They also identified two smaller isoforms that are derived by alternative splicing of hnRNP Q3. hnRNP Q2 lacks 34 amino acids from RRM2, and in hnRNP Q1, the last 74 amino acids from the C-terminus of hnRNP Q3 are replaced by the sequence VKGVEAGPDLLQ. All yeast two-hybrid clones identified in both studies contain the C-termini, including most of the RGG box, suggesting that the RGG box of hnRNP Q is necessary and sufficient for binding to SMN. This was confirmed by GST-pull down assays that identified an RG dipeptide-rich sequence from amino acids 518–549 as the minimal SMN-binding domain.

Although hnRNP R is mainly a nuclear protein, it was also found to colocalize with Smn in axons of motor neurons (Rossoll et al. 2002). hnRNP R has been shown to associate with the 3′ UTR of β-actin mRNA and to modulate localization of actin mRNA in neurites (Rossoll et al. 2003). While hnRNP Q3 has been isolated as a predominantly nuclear protein associated with the spliceosome complex (Neubauer et al. 1998), hnRNP Q1 has been

identified in several independent studies on various aspects of mRNA metabolism in the cytoplasm. Taken together, they suggest a role of hnRNP Q1 in mRNA transport, regulation of mRNA stability, and/or translation. Previously, hnRNP Q1 has been described as NSAP1, a protein interacting with the NS1 protein of minute virus of mice that is essential for viral replication in the cytoplasm (Harris et al. 1999). Regulation of mRNA stability by hnRNP Q1/NSAP1 was shown in a study on the major protein-coding-region determinant of instability (mCRD) of the c-fos proto-oncogene mRNA. hnRNP Q1/NSAP1 binds the mCRD and modulates the translationally coupled process of mRNA turnover. HnRNP Q1/NSAP overexpression stabilizes mCRD-containing mRNA by inhibiting deadenylation (Chen and Shyu 2003). In addition, hnRNP Q1/NSAP overexpression resulted in a dramatic stabilization of AU-rich element-containing mRNAs (Grosset et al. 2000). Work from several groups has demonstrated that hnRNP Q1/NSAP1 modulates cap-independent translation. It enhances IRES-dependent translation through interaction downstream of the hepatitis C virus polyprotein initiation codon (Kim et al. 2004) and it has also been shown to modulate IRES-dependent translation of cellular BiP mRNA through an RNA–protein interaction under heat stress conditions (Cho et al. 2007). Another substrate of hnRNP Q1 is mRNA related to the circadian rhythm. hnRNP Q1 has been shown to bind serotonin *N*-acetyltransferase (AANAT) both at its 3′ untranslated region to mediate mRNA degradation (Kim et al. 2005) and its 5′ region to regulate cap-independent translation (Kim et al. 2007). Other studies have connected hnRNP Q1 to vesicle transport and RNP transport granules. hnRNP Q1 has been described as SYNCRIP, a protein that interacts with the C2B domains of ubiquitous synaptotagmin isoforms; thereby, suggesting a potential link between vesicles and mRNA transport (Mizutani et al. 2000). hnRNP Q1/SYNCRIP has also been identified as a component of inositol 1,4,5-triphosphate receptor type I mRNA transport granules in rat hippocampal dendrites (Bannai et al. 2004). In another proteomic study, hnRNP Q1/SYNCRIP was isolated as a component of kinesin (KIF5)-containing RNA transport granules (Kanai et al. 2004). Therefore, it will be important to investigate whether SMN associates with hnRNP R/Q in neuronal processes, and whether this interaction is necessary for hnRNP R/Q mediated regulation of mRNA granules in neuronal processes.

4 A Role for SMN in Stress Granule Formation

Stress granules (SGs) are an additional type of RNA granule within neurons whose formation within the cell body and processes can be induced in response to low dose arsenate treatment (Vessey et al. 2006). In contrast to RNA transport granules, SGs have limited motility, are larger morphologically, and may assemble de-novo, or perhaps be converted from RNA transport granules (Kiebler and Bassell 2006; Anderson and Kedersha 2008). SGs serve as a depot to recruit mRNAs and translational factors (however, lacking some ribosomal components), where mRNAs are poised in translational arrest and also protected from degradation (Kedersha et al. 2005). A number of mRNA binding proteins are recruited to SGs in response to various stressors (Anderson and Kedersha 2006, 2008). The RGG box has been shown to be necessary for recruitment of some mRNA binding proteins to SGs (Yang et al. 2006). SMN was shown to target to SGs upon arsenate exposure and SMN-deficient fibroblasts from SMA patients showed impaired formation of SGs and recruitment of mRNAs (Hua and Zhou 2004b). The SG hypothesis to explain the neurodegeneration in SMA posits that impaired formation of SGs in axons, in response to various insults (i.e., oxidative stress), may lead to increased degradation of axonal mRNAs (Hua and Zhou 2004b). In that, many of the SMN-associated mRNA binding proteins are also localized to stress granules, where they play a role in mRNA stability regulation (Stohr et al. 2006), it will be important to assess how these interactions may affect the trafficking of mRNAs between various types of granules involved in mRNA transport, stability, and degradation, including stress granules and P-bodies (Kiebler and Bassell 2006). Collectively, these dynamics may have a great impact on mRNA translation in axons.

4.1 The SMN-mRNA Granule Hypothesis

As discussed earlier, a critical goal will be to continue to identify interactions between SMN and specific mRNA binding proteins that are localized to neuronal processes, and assess the function of these interactions in mRNA regulation. Although it has been shown that SMN can bind RNA in vitro, there is no evidence so far that it acts as an RNA-binding protein in vivo (Lorson and Androphy 1998; Bertrandy et al. 1999). SMN may impart binding specificity to RNA binding proteins through its role in the formation of the ribonucleoprotein complex. The past work on the SMN–Gemin complex and its role to facilitate Sm core assembly onto stem loops of snRNA may suggest a mechanism that could be used for mRNP assembly. Whether SMN interactions with mRNA binding proteins depend on Gemins will need to be addressed. Nonetheless, SMN interactions with mRNA binding proteins may modulate the binding of mRNA binding proteins to *cis*-acting elements and assembly of RNA transport granules. A model for the proposed role of SMN complexes in axonal RNA localization is shown in Fig. 2.

5 Animal Models of SMA

SMN is present in all metazoan cells with an evolutionary conserved role in small nuclear ribonucleoprotein assembly and pre-mRNA splicing. SMN orthologs have been disrupted in different genetic model organisms, ranging from yeast to mouse, in an effort to model the disease phenotype (Schmid and DiDonato 2007). These SMA models serve as powerful research tools to elucidate the pathogenic mechanism, and also to aid in the development of therapies for the treatment of spinal muscular atrophy. Using a variety of model organisms makes it possible to exploit their respective strengths and to validate results gained from different species. Analyzing the phenotype of these model organisms suggests a unique requirement for SMN orthologs in the development and maintenance of motor neurons (Monani et al. 2000a).

5.1 Caenorhabditis elegans

The nematode *Caenorhabditis elegans* has been used as a valuable research tool in many fields of biological research. Establishment of a cell lineage map, sequencing of the entire genome, and a well-characterized wiring diagram of its neurons allow an in-depth investigation of the development of its nervous system (Girard et al. 2007). CeSMN is an essential protein that is required for embryonic viability. Targeting *smn-1* with RNAi in all tissues (including maternal transcripts) and overexpression of the gene gives rise to locomotive defects and embryonic lethality (Miguel-Aliaga et al. 1999). Surviving hypomorphs demonstrate pleiotropic developmental defects in neuronal, muscular, and reproductive tissue that lead to locomotive defects, lack of muscle tone, vulval abnormalities, and sterility caused by a defect in germ cell maturation (Miguel-Aliaga et al. 1999).

Recently, a deletion mutant of *smn-1 (ok355)* has been characterized, which removes most of the gene including the translation start codon (Briese et al. 2009). Unlike the embryonic lethality caused by targeting *smn-1* with RNAi, maternally contributed CeSMN in the mutant allows for normal early larval development of *smn-1(ok355)* mutant animals, but causes late larval arrest, sterility, decreased lifespan, as well as impaired locomotion and pharyngeal pumping activity. The *smn-1(ok355)* phenotype can be partially rescued by neuronal, but not muscle-directed, expression of *smn-1*. A *C. elegans* ortholog of the human *Gemin2* gene and several RNA-binding proteins have also been identified as interaction partners of CeSMN (Burt et al. 2006). Knockdown of *smn-1* by RNAi, as well as deletion mutants have the potential to be utilized in screens searching for phenotypic modifiers as a further step toward potential SMA therapies (Briese et al. 2009).

5.2 Drosophila melanogaster

The fruit fly *Drosophila melanogaster* has proven to be an excellent invertebrate model organism for studying human neurodegenerative diseases. Mutational analyses and the existence of a broad array of screening techniques allow direct observation of the effects of the loss of gene function, making it an excellent in vivo system for the identification of genetic modifiers and the testing of therapeutic compounds (Lu and Vogel 2008).

The *Drosophila* genome bears a single copy of the *Smn* gene, which encodes a highly conserved homologue of SMN (DmSMN) (Miguel-Aliaga et al. 2000). *Drosophila* contains a remarkably simple SMN complex that lacks most Gemin proteins (Kroiss et al. 2008). This minimal complex mediates the assembly of spliceosomal snRNPs in a manner very similar to its vertebrate counterpart, by also preventing misassembly onto nontarget RNAs (Kroiss et al. 2008). Larval-lethal *Smn*-null mutations do not show detectable differences in spliceosomal snRNA levels, suggesting that these animals do not die from global snRNP depletion (Rajendra et al. 2007). Ectopic expression of human SMN exerts a dominant-negative effect that leads to developmental defects and pupal lethality, presumably, by forming a nonfunctional complex with endogenous DmSMN (Miguel-Aliaga et al. 2000). *Drosophila* mutant strains that contain point mutations in *Smn* similar to those found in SMA patients show abnormal motor behavior (Chan et al. 2003). Because of maternal contribution of the smn transcript, smn zygotic mutant embryos survive early development. Mutant larvae show severe motor abnormalities, defects in late developmental stages, and late larval lethality. Defects at the neuromuscular junction (NMJ) include disorganized synaptic motor neuron boutons, reduced clustering of the neurotransmitter receptor subunit GluR IIA, and reduced excitatory post-synaptic currents. Rescue experiments show that *Smn* gene activity is required in both neurons and muscle to alleviate this phenotype. Taken together, these studies point to a functional role of DmSMN at the NMJ (Chan et al. 2003).

Hypomorphic mutations in *Smn* with a tissue specific reduction of DmSMN protein levels in the adult thorax cause flightlessness and acute muscular atrophy (Rajendra et al. 2007). Mutant flight muscle motor neurons display motor axon routing and arborization defects coupled with a loss of the flight muscle-specific actin isoform Act88F expression. The authors also observed colocalization of DmSMN with sarcomeric actin, association between SMN and the thin filament crosslinker α-actinin, and disruption of α-actinin localization in the thoracic muscles of *Smn* mutants. These observations suggest a specific function for DmSMN in muscle and would underline the importance of this tissue in modulating the disease phenotype (Rajendra et al. 2007). However, it is not clear whether the defects observed in the *Smn* hypomorphs are caused by reduced DmSMN expression in the motor neurons, the thoracic muscles, or a combination of both.

Recently, a screen for genetic modifiers of an *Smn* phenotype, using the Exelixis collection of transposon-induced mutations, identified 17 enhancers and 10 suppressors (Chang et al. 2008). Among these were several genes not previously known to be associated with the genetic circuitry of *Smn*. These include several members of the bone morphogenic protein (BMP) signaling pathway. Interestingly, the observed NMJ defects in the SMA model can be ameliorated by increased BMP signaling, suggesting that drugs that stimulate BMP activity may provide potential SMA therapeutics (Chang et al. 2008).

5.3 Danio rerio

The zebrafish *Danio rerio* has been developed as an excellent vertebrate model organism for studying developmental biology and genetics (Beattie et al. 2007). A very stereotyped and well characterized neuromuscular system and external embryonic development allow for easy analysis of early nervous system development. Recent advances, such as large genetic

screens for mutations that disrupt development, the establishment of an effective antisense approach, and sequencing of the zebrafish genome, make them an ideal model system to study developing motor neurons and their axonal projections. Although targeted gene knockout is not yet established in the zebrafish model, most loss-of-function studies use a morpholino (MO) knockdown approach.

Beattie and colleagues have developed a zebrafish model for SMA, which demonstrate an axonal function for SMN. Using antisense morpholinos injected into zebrafish embryos at the 1–2 cell stage to reduce Smn levels lead to motor axon-specific pathfinding defects (McWhorter et al. 2003). While the number of motor neurons present at 24 h was not affected, motor axons were short or excessively branched. Importantly, specific reduction of Smn in individual motor neurons revealed that Smn is acting cell-autonomously. This study indicates an essential function of Smn in motor axon development in vivo, and suggests that these early developmental defects may contribute to later defects in synapse development that may precede motor neuron loss. To further characterize Smn functions necessary for motor axon outgrowth, *smn* MO were coinjected with various human *SMN* RNAs and assayed for their effect on motor axons (Carrel et al. 2006). Motor axon defects caused by Smn reduction were rescued by wild-type human SMN but not by SMN lacking exon-7, the predominant form in SMA, or other mutations in human SMN that also cause SMA. The identification of mutations that failed to rescue motor axon defects, but retained properties needed for snRNP assembly such as oligomerization and Sm protein binding, would indicate a critical function of SMN in motor axons that is relevant to SMA and is independent of snRNP biosynthesis (Carrel et al. 2006). SMNΔ7, SMN G279V, and SMN Y272C were defective in these properties and failed to rescue axonal defects. The human SMA mutant A111G was also unable to rescue motor axon defects while retaining Sm binding and oligomerization properties. In contrast, SMNΔ7 fused to an exon-7 sequence, containing the minimal cytoplasmic localization motif (QNQKE) (Zhang et al. 2007), was able to rescue motor axon defects, although it was deficient in oligomerization and Sm proteins binding in vitro (Carrel et al. 2006; Beattie et al. 2007). A synthetic point mutant of an exon-7 sequence (SMN Q282A) important for the cytoplasmic localization of SMN did not rescue the axon guidance defects, although this mutant retained snRNP function. Further work will be needed to show that this point mutant is fully competent for snRNP function, and it will be interesting if interactions with proteins other than Gemins are impaired. Such efforts to identify SMN domains that compromise SMN function in axon guidance, without affecting snRNP function, represent a useful approach toward identification of alternate mechanisms underlying SMA.

Another study used the morpholino knock-down approach to show an effect of SMN reduction on U snRNP metabolism (Winkler et al. 2005). Knockdown of SMN and the U snRNP assembly factors Gemin2 and pICln led to motor axon degeneration. This developmental defect was rescued by injection of purified U snRNPs into SMN- or Gemin2-deficient zebrafish embryos. These findings would imply that motor neuron degeneration in SMA patients may be a direct consequence of impaired production of U snRNPs (Winkler et al. 2005). However, these findings are contradicted by a study that did not find any aberrant motor axons caused by Gemin2 knockdown in morphologically normal embryos (McWhorter et al. 2008). Specific knockdown of Gemin2 in motor neurons by either injection into motor neurons or generation of genetic mosaics did not lead to motor axon defects. Since Gemin2 and SMN both function in snRNP biogenesis, yet, only SMN knockdown causes motor axon defects, these findings would support an axonal role for SMN independent of snRNP assembly. The authors have suggested that motor axon defects observed by Winkler et al. (2005) upon Gemin knockdown may be secondary to the overall morphological defects – including widespread defects in the nervous system – observed in these embryos.

5.4 Mouse Models of SMA

With the advent of gene targeting technology that is now routinely used to manipulate the genome, mouse models of human diseases have become the most widely used vertebrate animal models in biomedical research. Like all model organisms studied so far, mice have only a single copy of the SMN gene corresponding to human SMN1. As will be evident in the discussion below, mouse models of SMA have provided new insight into SMN function in synapse development. A list of commonly available SMA mouse models is shown in Table 2.

Mice that lack Smn due to homozygous insertion of a lacZ reporter gene after exon2a display massive cell death during early embryonic development and die during the morula stage prior to transition to the blastocyst stage and uterine implantation (Schrank et al. 1997). A follow-up study on Smn+/− heterozygous mice found a 46% reduction of Smn protein levels in the spinal cord and motor neuron degeneration resembling human type III SMA with normal lifespan and no reported overt phenotype (Jablonka et al. 2000). Between birth and six months of age, 40% of spinal cord motor neurons were lost relative to wild-type mice, but no further statistically significant loss occurred later. This order of pathophysiological events corresponds to an early phase of deterioration, followed by a phase of stabilization observed in SMA III patients (Crawford and Pardo 1996).

Two groups have chosen a very similar approach to model the SMA disease phenotype. These most commonly used SMA mouse models faithfully replicate the situation in human SMA patients by expressing human *SMN2* transgenes in an *Smn* knockout background. The Burghes lab used the knockout mouse from the Sendtner lab (Schrank et al. 1997) and inserted a 35.5 kb fragment containing only the *SMN2* transgene and its promoter region (*Tg(SMN2)89Ahmb*) (Monani et al. 2000b). The Li laboratory pursued a similar strategy by disrupting *Smn* exon 7 and using a larger 115 kb fragment of the human *SMN2* region that also contained flanking genes (*Tg(SMN2)2Hung*) (Hsieh-Li et al. 2000). *Smn* heterozygous mice, containing the transgene, were bred to give progeny lacking endogenous *Smn*, but carrying the respective transgene. Aside from minor differences between these transgenic models, both had strong SMA-like characteristics. Furthermore, in both models, the human *SMN2* transgene was able to complement the embryonic lethality of *Smn*−/− mice. The phenotype was modulated by the expression levels of *SMN2* and even rescued by sufficiently high copy number of the inserted *SMN2* transgene. The severely affected mice (*Smn*−/−, *SMN2*) have only one or two copies of the *SMN2* transgene on an *Smn* null background and frequently died perinatally. Surviving mice initially appeared normal but became progressively smaller and weaker than their normal littermates and presented with decreased mobility and suckling, labored breathing, and finally, limb tremor followed by death within days after birth. These mice produce low levels of SMN protein and suffered a 35–40% loss of spinal cord and lower-brainstem motor neurons by day five, despite normal numbers at birth. Primary embryonic motor neurons cultured from these mice showed defective axon outgrowth, but normal survival (Rossoll et al. 2003). Isolated sensory neurons were less severely affected (Jablonka et al. 2006). This is in agreement with a study on SMA patients, who underwent sural nerve biopsy that showed significant sensory nerve pathology in severely affected SMA type I patients, while no sensory nerve alterations were detected in SMA type II or III patients (Rudnik-Schoneborn et al. 2003). Taken together, these results suggest that motor neuron loss is a late event, occurring subsequent to axonal denervation (discussed below), and the timing of motor neuron loss is critical for disease severity. In both mouse models, expression from several copies of the *SMN2* transgene completely rescued the disease phenotype, with the exception of short, thick tails that became necrotic after about 2 weeks of age. The cause of this phenotype is currently unknown. A study with cultured primary motor neurons isolated from the *Smn−/−, SMN2* mice found defective accumulation and clustering of Ca_V 2.2 Ca^{2+} channels in the axonal

growth cones that was correlated with a reduction of local Ca^{2+} transients in distal axons and growth cones (Jablonka et al. 2007). The observed functional abnormalities of Ca^{2+} channels could contribute to maturation defects found in SMA.

Using the severe SMA models as a basis, several groups have inserted additional transgenes to modify the disease severity and answer specific questions about SMN function. Studies in *Drosophila* suggested that expression of *SMN* in both muscle and nervous tissue was required for complete rescue of an SMA phenotype (see above), implying that high levels of *SMN* expression in muscle may impact the SMA phenotype. To investigate whether this is also the case in severe mouse models of SMA, *SMN* was expressed in skeletal muscle fibers under the human skeletal actin (HSA) promoter and in neurons under the prion promoter (PrP) (Gavrilina et al. 2008). The results suggest that only neuronal *SMN* expression corrected the severe SMA phenotype in mice, whereas high *SMN* levels restricted to skeletal muscle fibers alone had no impact on the SMA phenotype or survival. It should be noted, however, that the transgene expressed from the PrP promoter showed “leaky” expression in skeletal muscle. Although it is possible that the timing and protein levels expressed from the HSA promoter was not sufficient to have an effect on the SMA genotype, an HSA-SMN strain that showed low levels of expression in the spinal cord and brain had significantly increased survival. Mice homozygous for PrP-SMN in an *Smn*−/−; *SMN2* background survived for an average of 210 days, and motor neuron counts in these mice were normal. This demonstrates that increased levels of full-length *SMN* in neurons, but not muscle, were required to correct the SMA phenotype.

Expression of a mutant SMN transgene harboring the A2G SMA mutation in a severe SMA background (*Smn*−/−; *SMN2*) delayed the onset of motor neuron loss, resulting in mice with symptoms and neuropathology similar to patients afflicted with SMA type III. These mild SMA mice presented with motor neuron degeneration, muscle atrophy, and abnormal electromyographs, and lived to about 8 months of age (Monani et al. 2003). In the absence of the SMN2 gene, the mutant SMN A2G transgene did not rescue the embryonic lethality, suggesting that low levels of wild-type SMN are required for the formation of functional SMN complexes.

To investigate motor axon development and innervation in severe SMA mice, the HB9:GFP transgene was introduced into the *Smn*−/−; *SMN2* +/+ strain by cross breeding to specifically label motor neurons with GFP (McGovern et al. 2008). Motor neurons innervating various muscles were examined from embryonic day 10.5 to postnatal day 2 for abnormalities in axon formation and outgrowth, but no defects were detected at any stage of development. However, a significant increase in unoccupied AChR clusters in intercostal muscles and the presence of axonal varicosities in motor axons in embryonic SMA mice indicated denervation during embryogenesis as one of the earliest detectable morphological defects in the SMA mice.

The question of whether SMNΔ7, the major splicing isoforms expressed from *SMN2* locus lacking exon 7, has a beneficial or detrimental effect has been answered unequivocally by several independent studies. Transgenic mice expressing *SMN*Δ*7* cDNA under the control of the SMN promoter were crossed onto a severe SMA background (*Smn*−/−; *SMN2*). Added expression of the *SMN*Δ*7* transgene (*Tg*(*SMN2***delta7*) *4299Ahmb*) turned out to be beneficial by extending mean survival of SMA mice homozygous for the targeted mutant *Smn* allele and homozygous for the two transgenic alleles from 5 to 13 days (Le et al. 2005). It is thought that expression of SMNΔ7 can rescue the SMA phenotype if a low level full-length SMN is present, probably by oligomerizing with full-length SMN and thus raising the effective concentration of SMN complexes (Le et al. 2005). In a detailed study of mice homozygous or hemizygous for the *SMN*Δ*7* transgene, the initial effects of reduced SMN

on the neuromuscular system were described as a NMJ synaptopathy (Kariya et al. 2008). The earliest structural defects appear distally prior to overt symptoms and involve the NMJ. Reduced levels of the SMN protein impair the normal maturation of acetylcholine receptor (AChR) clusters and lead to presynaptic defects, including poor terminal arborization and intermediate filament aggregation causing neurotransmission defects.

In a related study, the synaptic pathology at the neuromuscular junction in two different mouse models of SMA, the *Smn*−/−;*SMN2* mouse model of type I SMA and the *Smn*−/−;*SMN2;*Δ*7* mouse model, was analyzed in detail (Murray et al. 2008). NMJs in various muscles were disrupted in both mouse models, especially in postural muscles in the trunk. Loss of presynaptic inputs and nerve terminals with abnormal accumulations of neurofilament protein occurred even in early/mid-symptomatic animals in the most severely affected muscle groups. Skeletal muscles can be assigned to one of the two distinct classes of muscles, termed “fast synapsing” (FaSyn) and “delayed synapsing” (DeSyn) muscles, which differ in their pattern of neuromuscular synaptogenesis during embryonic development and maintenance of NMJs in adult mice (Pun et al. 2002). This study suggests that motor neurons with FaSyn-like, nonplastic characteristics may be more vulnerable to SMA-induced synapse loss than those with a DeSyn phenotype. Treatments that challenge synaptic stability result in nerve sprouting, NMJ remodeling, and ectopic synaptogenesis selectively on DeSyn muscles. Possibly, NMJs on FaSyn muscles lacking the synaptic plasticity are lost selectively and very early in SMA mice, suggesting that disease-associated motor neuron dysfunction may selectively fail to initiate maintenance processes at nonplastic FaSyn NMJs (Santos and Caroni 2003).

Electrophysiology and ultra-structure was the focus of a recent study on the function and structure of NMJ synapses in the *Smn*−/−;*SMN2;*Δ*7* mouse model of severe SMA (Kong et al. 2009). While NMJ synapses remained well connected late in the disease course, decreased density of synaptic vesicles and reduced probability of vesicle release at SMA NMJs was found to be associated with impaired morphological and biochemical maturation of postsynaptic NMJ terminals and myofibers. These findings suggest that NMJ synaptic dysfunction precedes axonal degeneration in severe SMA mouse models. Similar defects were described in an earlier investigation of the NMJ ultrastructure in SMA type 1 that showed shallow subsynaptic clefts and axon terminals devoid of transmitter vesicles (Diers et al. 2005).

A different approach for the generation of SMA mouse models was chosen in the Melki lab (Frugier et al. 2000). Mice that harbor a conditional allele of *Smn*, in which LoxP sites flank *Smn* exon 7 (SmnF7), allow conditional excision of Smn exon 7 when crossed with mice expressing Cre recombinase in a tissue-specific manner. Mice carrying a homozygous deletion of *Smn* exon 7 directed to neurons display progressive loss of motor axons, defects in NMJs, and massive accumulation of neurofilaments in terminal axons of the remaining neuromuscular junctions (Cifuentes-Diaz et al. 2002). Selective deletion of exon7 in either skeletal muscle (Cifuentes-Diaz et al. 2001) or liver (Vitte et al. 2004) also leads to severe defects in these tissues, confirming that full length *Smn* is essential for all tissues and cell types. The relevance of these findings for the disease phenotype of SMA is currently not clear. The observed defects differ from those seen in human SMA patients or the other mouse models that both retain at least some expression of full length SMN. It is likely that selective reduction of SMN levels under a certain threshold level in any tissue will lead to defects due to the essential function of SMN. Future studies are needed to investigate to what extent SMN deficiency in other tissues contributes to the SMA disease phenotype.

SMA mouse models have been crossbred with other mutant mice to study the effect of neuroprotective and anti-apoptotic mutations on the SMA disease phenotype. Slow

Wallerian degeneration (Wld^S) mice express a fusion of nicotinamide mononucleotide adenylyl transferase 1 (Nmnat-1) and ubiquitination factor e4b (Ube4b) that protects axons from Wallerian degeneration in genetic mutants such as the pmn mouse (Ferri et al. 2003). Wld^S in a SMA mouse model has no protective effect to alter the SMA phenotype, indicating that Wallerian degeneration does not directly contribute to the pathogenesis of SMA development (Rose et al. 2008; Kariya et al. 2009). Deletion of Bax, the major proapoptotic member of the Bcl-2 family, and overexpression of Bcl-xL, an anti-apoptotic of the Bcl-2 family, prevent neuronal cell death. Crossbreeding of Bax-deficient or Bcl-xL transgenic mice with SMA mice showed milder disease severity and longer life spans of the progeny as compared to their SMA littermates (Tsai et al. 2008). These results suggest that suppression of neuronal apoptosis has a potential to ameliorate the disease phenotype of SMA and may be a therapeutic target for future drug development.

Taken together, several recent studies on severe SMA mouse models emphasize early defects in NMJ synapses that include nerve terminal loss, neurofilament accumulation in presynaptic terminals, immature postsynaptic terminals, and functional abnormalities (Cifuentes-Diaz et al. 2002; Kariya et al. 2008; McGovern et al. 2008; Murray et al. 2008; Kong et al. 2009).

It will be important to compare the above phenotypes in animal models with observations from human samples. Likewise, we need to compare observations in human patients with the findings in model organisms. For example, in a recent study, SMA-I and SMA-II spinal cords showed a significant number of motor neurons that had migrated aberrantly toward the ventral spinal roots (Simic 2008; Simic et al. 2008). These heterotopic motor neurons (HMN) were located mostly in the ventral white matter and had no axon or dendrites. The authors propose that abnormal migration, differentiation, and lack of axonal outgrowth may induce cell death in displaced HMNs and contribute to the pathogenesis of SMA. It will be interesting to see whether similar phenotypes can be observed in animal models of SMA.

6 Therapeutic Strategies

SMA is the leading genetic killer of infants and toddlers with an incidence of 1/10,000 and a carrier frequency of one in 35 (Wirth et al. 2006). Currently, there is no cure for SMA or treatment to stop its progression. Treatment is symptomatic and supportive and includes treating pneumonia, curvature of the spine, and respiratory infections, if present (*http://www.ninds.nih.gov/disorders/sma/sma.htm*). The human toll of this disease, combined with its unique genetic profile, has focused attention from the NIH, industry, academia, and advocacy organizations on developing a treatment. SMA was selected in 2003 by the National Institute of Neurological Disorders and Stroke (NINDS) for a model translational research program aimed at accelerating drug discovery efforts against neurodegenerative diseases.

To uncover therapeutic options for SMA patients, research groups typically focus on one of the following strategies (Lunn and Stockwell 2005; Sumner 2006): (1) Elevation of endogenous full length SMN levels from the SMN2 gene by upregulating the SMN2 promoter, correcting alternative SMN2 pre-mRNA splicing, increasing read-through of SMN Δ 7 mRNA, stabilization of SMN protein, and modulating SMN2 translation; (2) Neuroprotection; (3) Delivery of SMN with viral vectors (gene therapy); and (4) Implantation of stem cell derived precursors into the spinal cord (stem cell therapy). Beyond the scope of this review, many drugs, including histone deacetylase inhibitors, such as valproic acid (VPA) or 4-phenyl-butyrate (PBA), have been shown to increase full length SMN2-derived RNA and protein levels in cell culture. None of these drugs is believed to act specifically on expression of the SMN gene, but is expected to change the expression level

of hundreds of genes. Clinical trials are underway to investigate the effect of VPA, PBA, and other drugs on motor function in SMA patients (Lunn and Wang 2008; Oskoui and Kaufmann 2008). Also, beyond the scope of this review, many different approaches have been used successfully to specifically restore inclusion of exon7 in *SMN2* mRNA in vitro by using small antisense RNA molecules that modulate exon7 inclusion by directly binding to *SMN2* transcripts (Madocsai et al. 2005; Coady et al. 2007; Marquis et al. 2007; Singh 2007; Hua et al. 2008). While this approach holds a lot of promise, it remains to be seen whether such therapeutic oligonucleotides can be successfully delivered to spinal motor neurons.

6.1 Gene Therapy

Multiple single injections of a lentiviral vector construct that was pseudotyped with rabies virus glycoprotein in various muscles of SMA mice restored SMN expression to motor neurons, reduced motor neuron death, and increased the life expectancy by an average of 5 days, compared with untreated animals (Azzouz et al. 2004). Recently, adeno-associated virus (AAV) 9 injected intravenously has been shown to bypass the blood–brain barrier and efficiently target cells of the central nervous system (Foust et al. 2009). Intravenous injection of AAV9-GFP into neonatal mice through the facial vein resulted in extensive transduction of dorsal root ganglia and motor neurons throughout the spinal cord. These promising results suggest that lentiviral or AAV based vectors may enable the future development of gene therapies for SMA.

6.2 Stem Cells

Stem cells can be utilized as a biological tool to examine the abnormal cellular and molecular processes in these cells to gain a better understanding of the SMA pathophysiology. In a recent study, neurosphere-derived neural stem cells from the severe SMA mice (*Smn−/−;SMN2*) showed differences in proliferation and differentiation as compared to wild-type controls (Shafey et al. 2008). Creating a cell-culture model of human disease can also be a useful tool in developing screens for potential therapeutic agents. Human ES cell-derived neuroprogenitor cells have been generated as a new primary cell source for the purpose of developing assays for new SMA therapeutics (Wilson et al. 2007). Endogenous stem cells present in the mammalian nervous system may be manipulated to induce neural tissue repair. Alternatively, neuronal progenitor cells derived from stem cells that are committed to the motor neuron lineage can be transplanted into the injured nervous system as a therapeutic strategy. Transplanted cells may contribute to the repair of the damaged neuronal circuits, not only by directly replacing degenerated cells, but also by providing support in many other ways (Nayak et al. 2006).

In a study on paralyzed rats, the transplantation of ES cells that were treated to induce differentiation into motor neurons has shown promising results (Deshpande et al. 2006). Multipotent neural stem cells isolated from the spinal cord of GFP-expressing mice that were also primed for differentiation into motor neurons were transplanted intrathecally into SMA mice (*Smn−/−; SMN2+/+; SMN* Δ *7+/+*) (Corti et al. 2008). Treated mice showed improved motor function and extended survival from 13 to 18 days. Transplanted cells even generated a small number of motor neurons that were properly localized to the ventral horn of the spinal cord, but secretion of trophic factors from the transplanted cells may also be responsible for the observed effect (Corti et al. 2008). Stem cell transplantation, combined with other molecular and pharmacologic approaches, may be a promising strategy in the development of SMA therapies.

An especially promising resource to develop an in vitro SMA disease model and develop novel therapies is based on two recent developments: (1) the generation of induced

pluripotent stem (iPS) cells from patient dermal fibroblasts (reviewed by (Lowry and Plath 2008), and (2) the differentiation of stem cells into motor neurons (Wichterle et al. 2002). A combination of these approaches was used to generate ALS patient-specific motor neurons (Dimos et al. 2008). Recently, these two combined approaches were used in a study to generate iPS cells from skin fibroblast samples taken from a child with SMA (Ebert et al. 2008). These cells were differentiated into motor neurons that showed selective deficits compared to those derived from the child's unaffected mother. Initially, iPS-SMA cells produced similar numbers of motor neurons, but showed a decline at later time points. Diffuse synapsin staining suggested specific presynaptic maturation deficits in the iPS-SMA cultures. These results validate motor neurons derived from iPS cells as a promising new in vitro model for studying the SMA disease mechanism and drug discovery.

7 Open Questions and Perspectives

Despite recent progress in research of SMN function and the pathophysiology of SMA, many open questions remain that have important implications for SMA therapy, and are considered below.

Does a defect in the canonical housekeeping function of SMN in snRNP assembly lead to SMA?

While it is known that extremely low SMN levels affect splicing, it is not clear yet, whether a more moderate reduction, as seen in patients, cause splicing defects in motor neurons that may explain the disease phenotype. Identifying aberrantly spliced transcripts with arrays and other methods will be required to answer this question.

Does SMN play a role in the assembly of neuronal RNP complexes?

While there is evidence that SMN affects local β-actin mRNA levels in axons, it will be interesting to see whether other mRNAs are targets of SMN complexes. While SMN is transported in axons and co-localizes with interacting proteins, the composition of these putative RNA granules is not known. Proteomic studies of RNA granules to date have not identified SMN as a component. It will be important to further characterize a putative function of SMN in the assembly of axonal RNP complexes and to assess its role in the pathogenesis of SMA.

Are motor neurons the only cells affected by SMN deficiency?

The question whether SMA is caused by SMN deficiency in neurons, muscle fibers, or both appears still controversial. Most current studies suggest that low levels of SMN in motor neurons lead to SMA. It is important to clarify whether upregulation of SMN function in motor neurons is necessary and sufficient to prevent SMA. Presently, it cannot be excluded that other cells in the circuitry of the spinal cord or muscle tissue contribute to the pathophysiology of SMA. Indeed, more broadly restored SMN expression throughout the neuromuscular system, including neurons and muscle, may be necessary.

Which other genetic modifiers affect the disease phenotype of SMA?

A study in SMA discordant families with affected and asymptomatic siblings carrying the same *SMN1* mutations has identified PLS3 as a modifier of SMA severity (Oprea et al. 2008). It would be interesting to see whether expression of a PLS3 transgene in spinal cord or other tissues can indeed rescue the SMA phenotype in mouse models of SMA. Comparisons across several animal models will help to further characterize these targets and assess epistatic relationships of modifiers, especially, to conduct genetic modifier screens in invertebrate animals, such as *Drosophila* (Chang et al. 2008) and *C. elegans*, which may lead

to the identification of pathways that modulate the SMA phenotype and the discovery of novel therapeutic targets.

When do first neuromuscular defects develop, and can further progression be stopped?

Studies in several animal models of SMA point to early developmental defects of motor axons and NMJs and a dysfunction of motor neurons preceding cell death. The interaction of motor axons with muscle fibers in neuromuscular synapses is an important distinctive feature that distinguishes the motor neuron from all other neurons, which may underlie its specific vulnerability. It will be important to investigate how early developmental defects influence the establishment and maturation of muscle end-plate innervation patterns and may set the stage for subsequent denervation and eventual motor neuron cell death (Hausmanowa-Petrusewicz and Vrbova 2005). A question that is highly relevant for therapeutic intervention is the time window during which treatment, such as raising SMN levels, will benefit patients. The generation of conditional mouse models that allow for the induced expression of SMN at various time points (e.g., via tetracycline or tamoxifen) or conditional alleles that are engineered to revert to fully functional SMN via Cre-mediated recombination will be required to answer that question. Studies in several severe SMA models suggests that low SMN levels may lead to defects very early in development, at least in severe SMA type I, which has important implications for the development of a therapy for SMA. If an effective SMA drug treatment becomes available, intervention at a presymptomatic stage will require the establishment of neonatal screening to identify children at risk to develop SMA.

Results derived from addressing these questions will ultimately aid in the development of therapies for the treatment of SMA. Since axonal transport defects are common to many neurological disorders, including Alzheimer disease (AD), Huntington disease (HD), amyotrophic lateral sclerosis (ALS), and other forms of motor neurons disease, elucidating the cause of axonal defects in SMA may have widespread applicability to other types of neurological diseases (Duncan and Goldstein 2006).

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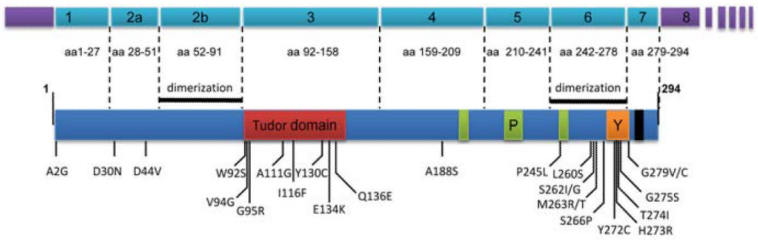

Fig. 1.

SMN1 exon boundaries and protein domain structure. *Top*: SMN1 is encoded by 9 exons. Coding regions are indicated in *cyan* and untranslated regions in *purple*. *Bottom*: Domains required for oligomerization of SMN are indicated as *black bars*. Other regions of interest are the Tudor domain (*red*), poly-proline regions (*green*), the YG box (*orange*), and the cytoplasmic targeting motif (*black*). Point mutations identified in *SMN1* genes of SMA patients are indicated below (Wirth 2000; Alias et al. 2008)

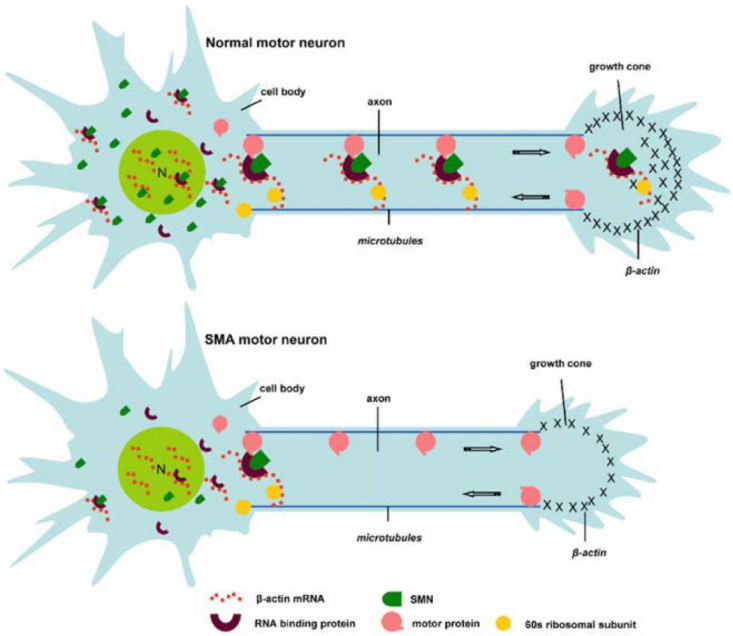

Fig. 2.

Model of a putative function of SMN complexes in axonal RNA localization. Data from different labs suggest a model whereby SMN-associated RNP complexes contribute to the localization of β-actin mRNA and probably other transcripts. SMN may facilitate the assembly of RNA cargo molecules with different RNA-binding proteins, adaptor proteins, molecular motor proteins, translational components, and auxiliary factors required for efficient transport and/or local translation (Based on a model by Lei Xing.)

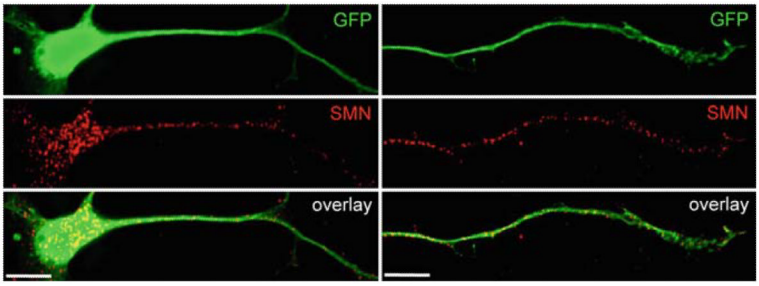

Fig. 3.

SMN-containing granules are transported along motor axons. SMN is localized in granules that are actively transported into neuronal processes and growth cones (Zhang et al. 2006). Shown above are murine primary embryonic motor neurons expressing GFP from the motor neuron-specific HB9 promoter. SMN-containing granules stained with specific antibodies are present in the cell body, dendrites, and axon, including the growth cone (*red*). Size bar = 10 μm (photomicrograph provided by Lei Xing)

Table 1

SMN-associated proteins, functions, and motifs[a]

SMN associated protein	Function	Motifs[b]	References
SMN-Gemin complex			
Gemin1/SMN	snRNP assembly	Tudor, Y/G box	Liu et al. (1997)
Gemin2/SIP1	snRNP assembly	–	Liu et al. (1997)
Gemin3/DDX20/DP103	snRNP assembly	DEXDc, HELICc	Charroux et al. (1999); Meister et al. (2000)
Gemin4/GIP1	snRNP assembly	Leu zipper	Charroux et al. (2000); Meister et al. (2000)
Gemin5/p175	snRNP assembly	WD40 repeats	Gubitz et al. (2002)
Gemin6	snRNP assembly	–	Pellizzoni et al. (2002a)
Gemin7/SIP3	snRNP assembly	RG-rich	Baccon et al. (2002)
Gemin8	snRNP assembly	–	Carissimi et al. (2006)
unrip	snRNP assembly	WD40 repeats	Meister et al. (2001)
snRNP core proteins			
Sm proteins/Sm B/B–, D1–3,E,F,G	snRNP assembly	Sm domain, RG rich	Liu et al. (1997); Friesen and Dreyfuss (2000)
Sm-like proteins/Lsm 10,11	snRNP assembly	Sm domain, RG rich	Friesen and Dreyfuss (2000); Brahms et al. (2001)
snoRNP core proteins			
Fibrillarin/FBL	snoRNP assembly	RGG box, RNP-2	Liu and Dreyfuss (1996); Jones et al. (2001); Pellizzoni et al. (2001a)
GAR1/Nola1	snoRNP assembly	RGG box	Pellizzoni et al. (2001a)
snRNP import			
Snurportin and importin β	Nuclear import of snRNPs	HEAT repeat	Narayanan et al. (2002)
TGSI (trimethylguanosine synth. 1)	Nuclear import of snRNPs	–	Mouaikel et al. (2003)
Cajal body/coiled body			
Coilin/p80	Recruitment of SMN to Cajal bodies	–	Hebert et al. (2001)
Transcription			
mSin3A	Transcriptional regulation	PAH, HDAC interact	Zou et al. (2004)
EWS (Ewing Sarcoma)	Transcriptional regulation	RGG box	Young et al. (2003)
Viral proteins			
Papilloma virus E2	Transcriptional regulation	–	Strasswimmer et al. (1999)
Epstein-Barr virus nuclear antigen	Transcriptional regulation	–	Barth et al. (2003)
Minute virus NS1 and NS2	Viral replication and transcriptional regulation	–	Young et al. (2002b)
Actin metabolism			
Profilin	Control of actin dynamics	–	Giesemann et al. (1999b)
T-Plastin/PLS3	Actin bundling	EFh, CH	Oprea et al. (2008)
Apoptosis			
p53	Apoptosis	–	Young et al. (2002a)
Bcl-2	Antiapoptosis	BH4, BCL	Iwahashi et al. (1997)

SMN associated protein	Function	Motifs[b]	References
RNA metabolism			
hnRNP U	RNA metabolism	SAP, SPRY, RGG	Liu and Dreyfuss (1996)
hnRNP Q and R	RNA metabolism	RRM, RGG	Mourelatos et al. (2001); Rossoll et al. (2002)
FUSE-binding protein/FBP	RNA metabolism	KH	Williams et al. (2000b)
KSRP/FBP2/ZBP2/MARTA1	RNA metabolism	KH	Tadesse et al. (2008)
FMRP	RNA metabolism	KH, Agenet	Piazzon et al. (2008)
U1A	pre-mRNA splicing	RRM	Liu et al. (1997)
Galectin 1 and 3/LGALS1	pre-mRNA splicing	GLECT	Park et al. (2001)
RNA helicase A	Transcription	DSRM, DEXDc, HELICc	Pellizzoni et al. (2001b)
RNA polymerase II	Transcription	–	Pellizzoni et al. (2001a)
Rpp20 (Ribonuclease P 20 kDa subunit)	tRNA and rRNA metabolism	–	Hua and Zhou (2004a)
Nucleolin and B23	rRNA metabolism	RRM, RGG-box	Lefebvre et al. (2002)
ISG20 (Interferon stimul. gene 20)	Degradation of ssRNA	EXOIII	Espert et al. (2006)
TIAR (TIA-1-related protein)	Stress granule formation	RRM	Hua and Zhou (2004b)
TDP-43 (TAR DNA-binding protein)	mRNA splicing and transcription	RRM	Wang et al. (2002)
NFAR-1/2/nuclear factor associated with dsRNA	RNA metabolism	DZF, DSRM	Saunders et al. (2001)
Others			
OSF (osteoclast-stimulating factor)	Src-related signaling	SH3, ANK	Kurihara et al. (2001)
USP9X (Ubiquitin-specific protease 9)	Deubiquitylating enzyme	–	Trinkle-Mulcahy et al. (2008)
PPP4 (protein phosphatase 4)	Ser/Thr protein phosphatase	PP2Ac	Carnegie et al. (2003)
FGF-2 (fibroblast growth factor 2)	Growth factor	FGF	Claus et al. (2003)
hsc70 (heat shock cognate prot. 70)	Protein folding/trafficking	–	Meister et al. (2001)
ZPR1 (zinc-finger protein 1)	Protein translation?	Zpr1	Gangwani et al. (2001)
BAT3/HLA-B assoc. transcript 3[c]	Apoptosis/regulation of p53	UBQ domain	Stelzl et al. (2005)
UNC119/HRG4[c]	Photoreceptor synaptic protein	GMP-PDE delta	Stelzl et al. (2005)
RIF1 (receptor interact. factor 1)[c]	Transcriptional repressor	–	Stelzl et al. (2005)
GDF9 (growth diff. factor 9)[c]	Growth factor	TGFB	Stelzl et al. (2005)
COPS6 (COP9 signalosome subunit 6)[c]	Regulation of ubiquitin ligases	JAB/MPN	Stelzl et al. (2005)
Leukocyte receptor cluster LRC member 8[c]	–	SAC3/GANP	Rual et al. (2005)
FLJ10204/C8orf32[c]	–	–	Rual et al. (2005)

[a] Meister et al. (2002); Peri et al. (2003); Stark et al. (2006); Wirth et al. (2006)

[b] Based on Schultz et al. (1998)

[c] Interaction partners identified in high throughput screens may have not been confirmed by independent methods

Table 2

SMA mouse models available from The Jackson Laboratories

Stock	Strain genotype	Average survival	References
006214	*FVB.Cg-Smn1$^{tm1Msd/J}$*	90–100 h	Schrank et al. (1997)
005024	*FVB. Cg-Tg(SMN2)89Ahmb Smn1$^{tm1Msd/J}$*	0–5 days[a]	Monani et al. (2000b)
006570	STOCK Smn1^{tm1Msd} Tg(Hlxb9-GFP) 1Tmj Tg(SMN2)89Ahmb/J	0–5 days[a]	McGovern et al. (2008)
005025	*FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1$^{tm1Msd/J}$*	ca. 13 days	Le et al. (2005)
005026	*FVB.Cg-Tg(SMN2)89Ahmb Tg(SMN1*A2G)2023Ahmb Smn1$^{tm1Msd/J}$*	<1 year[a]	Monani et al. (2003)
006146	*B6.129-Smn1$^{tm1Jme/J}$*	Normal[b]	Frugier et al. (2000)
006138	*FVB.129(B6)-Smn1$^{tm1Jme/J}$*	Normal[b]	Frugier et al. (2000)
005058	FVB.Cg-Tg(SMN2)2Hung Smn1^{tm1Hung}/J	<P10–normal[c]	Hsieh-Li et al. (2000)

[a] Difference between the survival times reported in the original publication and the survival of the strains available from The Jackson Laboratory may be due to inbreeding (http://jaxmice.jax.org/list/ra1733.html). It is the experience at The Jackson Laboratory that mice hemizygous for the *SMN2* transgene do not survive

[b] When crossed to a strain expressing Cre recombinase in a conditional manner, this mutant mouse strain will develop SMN-deficiency and associated defects in the affected tissue

[c] Average time of survival depends on the transgene copy number