Is spinal muscular atrophy the result of defects in motor neuron processes?

Michael Briese,[1] Behrooz Esmaeili,[1,2] and David B. Sattelle[1]*

Summary

The hereditary neurodegenerative disease spinal muscular atrophy (SMA) with childhood onset is one of the most common genetic causes of infant mortality. The disease is characterized by selective loss of spinal cord motor neurons leading to muscle atrophy and is the result of mutations in the survival motor neuron (*SMN*) gene. The SMN protein has been implicated in diverse nuclear processes including splicing, ribosome formation and gene transcription. Even though the genetic basis of SMA is well understood, it is not clear how defects in these ubiquitous processes result in motor neuron degeneration leaving other tissues unaffected. Recent evidence from animal and cell culture models of SMA points to roles for SMN in neurite outgrowth and axonal transport. Disruption of these functions might be particularly detrimental to motor neurons given their high metabolic demands and precise connectivity requirements, thus providing a possible explanation for the specificity of motor neuron susceptibility in SMA. Understanding the molecular mechanisms of SMN activity in neuronal processes may generate new targets for future therapeutic strategies. *BioEssays* 27:946–957, 2005.

[1]MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, Oxford, UK.
[2]Genetics Unit, Department of Biochemistry, University of Oxford, Oxford, UK.
Funding agency: Michael Briese is supported by a Wellcome Trust 4 Year Doctoral Studentship in Neuroscience.
*Correspondence to: David B. Sattelle, MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.
E-mail: david.sattelle@human-anatomy.oxford.ac.uk
DOI 10.1002/bies.20283
Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: hnRNP, heterogeneous nuclear ribonucleoprotein; NMJ, neuromuscular junction; SMA, spinal muscular atrophy; SMN, survival motor neuron; SMNΔ7, SMN lacking exon 7; UsnRNP, uridine-rich small nuclear ribonucleoprotein; UTR, untranslated region; ZBP, zipcode-binding protein.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by loss of lower motor neurons in the anterior horn of the spinal cord, accompanied by progressive wasting of associated muscles and, ultimately, paralysis. Based on time of onset of the disease and its severity, SMA can be subdivided into three types with onset during childhood (I-III), one type with adult onset (Type IV) and very severe cases with prenatal onset and early neonatal death, classified as Type 0 (Table 1).[1–3] Type I SMA (Werdnig-Hoffmann disease) is characterized by an early onset of general muscle weakness before or around six months. Affected children are never able to walk or sit and have an average lifespan of 8 months. Children with less severe forms of SMA generally survive beyond two years and are capable of unaided sitting (Type II) or standing and walking (Type III or Kugelberg-Welander disease). Patients with adult-onset SMA (Type IV) develop their first clinical symptoms over the age of 30.[4]

The SMA-determining gene, the survival motor neuron (*SMN*) gene (Fig. 1), was mapped to chromosome 5q13.[5,6] Lefebvre et al.[7] subsequently showed that in humans the *SMN* gene is present as a telomeric copy (*SMN1*) and an inverted centromeric copy (*SMN2*), which is thought to have arisen by intrachromosomal genomic duplication.[8] Comparative sequence analysis revealed that the SMA region in 5q13 is gene-rich and highly unstable having undergone extensive segmental inter- and intrachromosomal duplications.[8] The *SMN2* gene differs functionally from *SMN1* by a C/T transition at position +6 in exon 7, which creates an exonic splicing silencer promoting repression of exon 7 splicing.[9] Therefore, the majority of *SMN2* transcripts give rise to an unstable protein product lacking the 16 amino acids encoded by exon 7, although a small amount of transcript retaining exon 7 remains and generates functional SMN.[10]

Most patients with childhood-onset, recessively-inherited SMA have mutations in the *SMN1* gene. The vast majority of SMA cases (>95%) can be attributed to deletions or conversions involving *SMN1* exon 7.[7,11] Since *SMN2* produces functional SMN protein only at low levels, it cannot fully compensate for the homozygous loss of *SMN1*. However, the

Table 1. Diagnostic features in the classification of spinal muscular atrophy

Type	Age at onset	Motor abilities
0	Prenatal onset	Reduced fetal movements *in utero*; some degree of spontaneous ventilation and movement at birth
I	At birth or <6 months	Never able to sit
II	<18 months	Unable to stand or walk unaided
III	>18 months	Able to walk unassisted
IV	Adult onset	Mild proximal muscle weakness

SMN2 copy number seems to have an effect on the SMA phenotypic severity as patients with type II or type III SMA tend to have more *SMN2* copies than type I patients.[12,13] Thus, the inverse correlation between *SMN2* gene copy number and disease severity suggests that SMA can be attributed to a dose effect of SMN with *SMN2* being a modifying gene due to the small amount of functional SMN it generates.[14] Adult-onset proximal SMA (type IV) seems to be genetically heterogeneous with some but not all patients showing deletions in the *SMN* gene.[15–17]

The functions of the SMN protein in nuclear processes, in particular its role in the assembly of RNA–protein complexes involved in splicing, have been extensively reviewed.[18–21] More recently, this ''nucleocentric''[22] view of SMN has been challenged by discoveries of SMN activities in the neuronal periphery, i.e. in cell processes. These results may generate novel ideas on the cascade of pathological events leading to SMA and may reveal common links to other neurodegenerative diseases.[23,24] Here we focus attention on recent evidence for possible roles of SMN in neurite outgrowth, axonal transport and synaptic function.

The nucleocentric view of SMA

The *SMN* gene is evolutionarily highly conserved, being detected in yeast[25] and diverse animals ranging from vertebrates to invertebrates, indicating an essential function of the SMN protein.[26] Studies investigating the structure, molecular characteristics and interaction partners of SMN have pointed towards vital roles in nuclear processes such as splicing and gene transcription.

SMN functions in splicing-related processes

The SMN protein oligomerizes and associates with Gemins 2–7 to form a macromolecular assembly machine, the 'assemblysome'.[19,27,28] This SMN protein complex assists in the assembly of many different classes of ribonucleoproteins (RNPs) that function in RNA splicing, ribosomal production and gene transcription. SMN exists in nuclear foci termed gems ('gemini of coiled bodies'),[29] whose function is presently unknown. It is also found in the cytoplasm where it is involved in the biogenesis of small nuclear RNPs (snRNPs), in particular uridine-rich snRNPs (UsnRNPs), which are the principal components of the spliceosome. Assembly of UsnRNPs, the most common of which are U1, U2, U4, U5 and U6, is a multistep process and critically depends on the activity of the SMN complex (Fig. 2). The main function of SMN in UsnRNP biogenesis involves the activation of seven spliceosomal Sm proteins and their assembly as the Sm core onto oligo-uridine stretches of UsnRNAs. There is evidence that the SMN complex remains bound to UsnRNPs after Sm core formation and that nuclear import of UsnRNPs is SMN complex-dependent.[30]

How does SMN mediate selective Sm core assembly only on UsnRNAs? A number of studies have shed light on the specificity of Sm core formation. For example, Pellizzoni et al.[31] reported that, in the absence of SMN, the Sm proteins assemble non-specifically on rRNA and tRNA in vitro. This result suggests that SMN is required to prevent non-selective targeting of Sm proteins. Also, Yong et al.[32] detected sequence elements and structural features in UsnRNAs to which the SMN complex binds to with high affinity. Thus, the SMN complex seems to facilitate UsnRNA-specific Sm core formation in two ways: (i) by identifying specific features of UsnRNAs, which ensures correct targeting of the Sm core and (ii) by sequestering of Sm proteins, through binding to their

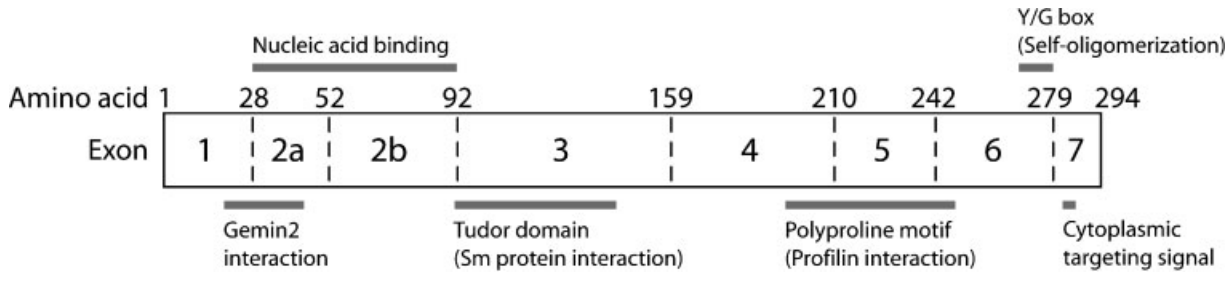

Figure 1. Schematic diagram of SMN showing coding exons and the relative localization of selected domains with known functions. In *SMN2* exon 7 is spliced out resulting in a truncated protein lacking the C-terminal-most 16 amino acids.

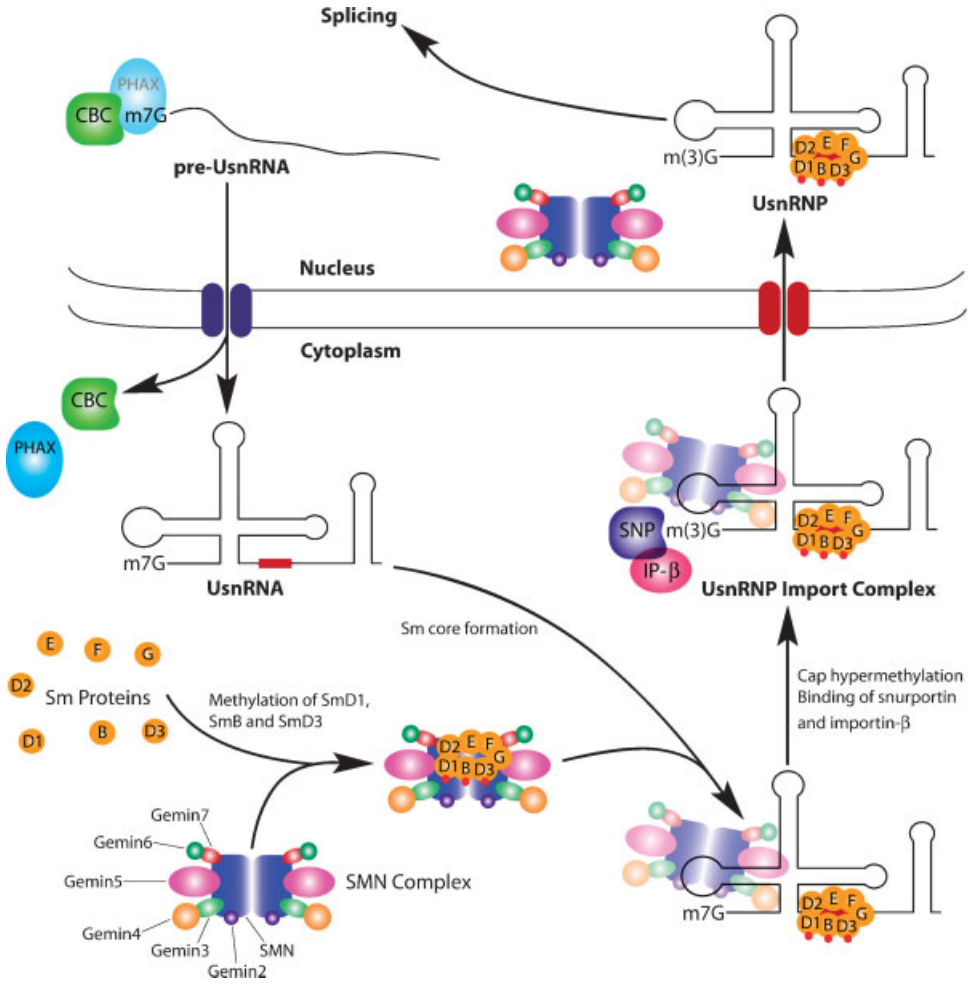

Figure 2. SMN functions in the biogenesis of uridine-rich small nuclear ribonucleoproteins (UsnRNPs). Following their transcription by RNA polymerase II, pre-UsnRNAs are capped at the 5′ end with 7-methylguanylate (m7G). This cap structure is recognized by the heterodimeric nuclear-cap-binding complex (CBC) composed of CBP20 and CBP80, and the export adaptor protein PHAX.[94,95] Further maturation of UsnRNAs takes place in the cytoplasm and involves the assembly of seven Sm proteins, which bind to oligo-uridine stretches (red box) with high affinity, on the UsnRNAs to form the Sm core. This step is mediated by the cytoplasmic SMN complex, composed of oligomeric SMN and Gemins2–7, which pre-assembles the Sm proteins before transferring them onto UsnRNAs in an ATP-dependent manner.[31,96] Sm proteins interact with SMN preferentially via protein domains rich in arginine and glycine (RG).[97] Dimethylation of arginine residues in these RG-rich domains greatly enhances their affinity for SMN which has been observed for the Sm proteins D1, D3 and B.[98,99] These Sm proteins are methylated by an arginine methyltransferase (methylosome) prior to their association with the SMN complex.[100] Following their assembly by the SMN complex, the seven Sm proteins are transferred onto UsnRNAs. The SMN complex itself was found to have an intrinsic affinity for UsnRNAs and remains bound to UsnRNPs even after Sm core formation. In U1snRNA, for example, the 5′-most stem–loop structure was identified as the high-affinity binding site for SMN.[101] After Sm core formation, the UsnRNP 5′ cap becomes hypermethylated (m(3)G) which forms a recognition motif for snurportin (SNP). The latter, together with importin β (IP-β), mediates transfer of the mature UsnRNP into the nucleus. SMN is part of this UsnRNP import complex containing snurportin and importin β and is required for the transport of UsnRNPs into the nucleus.[30,102,103] Following their re-import into the nucleus, the mature UsnRNPs function in splicing of pre-mRNAs.

arginine- and glycine-rich domains, thereby reducing the freely available amount of Sm proteins.

Do defects in splicing cause SMA?

Even though the role of SMN in snRNP biogenesis is well studied, it remains unclear whether there is a causal link between defects in this function and SMA pathogenesis. It has been suggested that reduction of SMN leads to defects in the production of spliceosomal snRNPs in the cytoplasm and/or in reactivation of spliceosomal complexes in the nucleus.[33] Such disruption of the splicing machinery may result in incorrectly spliced mRNAs encoding vital, motor neuron-specific

proteins, the loss of which leads to motor neuron degeneration. Pellizzoni et al. observed that wild-type SMN, but not mutant SMN forms harbouring SMA-causing mutations or deletions, shows a strong stimulatory effect on pre-mRNA splicing in an in vitro system indicating a role for SMN in the splicing cycle.[33]

To determine whether defects in snRNP biogenesis correlate with motor neuron loss in SMA Jablonka et al.[34] generated double heterozygous mice deficient for *Smn*, which is the murine homologue of human *SMN*, and *Gemin2*, the products of which are components of the snRNP assembly-some. Compared to $Smn^{+/-}$ mice the $Smn^{+/-}$;$Gemin2^{+/-}$ mice show a reduction of Gemin2 but not of Smn. Importantly, the double heterozygous deficient mice show a significantly reduced number of lumbar motor neurons compared to $Smn^{+/-}$ mice. This result shows that the extent of motor neuron degeneration caused by low levels of the murine Smn protein can be artificially potentiated by downregulation of Gemin2. The authors also demonstrated that, in $Smn^{+/-}$;$Gemin2^{+/-}$ mice, the nuclear pool of Sm proteins is reduced indicating a defect in spliceosomal UsnRNP assembly. Further support for the hypothesis that defects in snRNP biogenesis are relevant to the SMA pathogenesis derives from the observation that the commonest SMA-causing non-deletion mutations in SMN abolish its ability to interact with Sm proteins and Gemins and affect its tendency to oligimerize.[35,36] These mutations include E134K within the Tudor domain, Y272C, which is the most common subtle mutation causing SMA,[37] substitutions in exon 6 and deletion of exon 7.

These data, however, do not yet provide conclusive evidence that there is a link between defects in pre-mRNA processing and the SMA pathology. As an example, Jablonka et al.[38] investigated the effects of Smn reduction in mice on splicing of the apoptotic regulator *Ich-1*, also known as *caspase-2*. The *Ich-1* gene is expressed at high levels during embryogenesis in motor neurons and, due to alternative splicing, produces both a pro-apoptotic and an anti-apoptotic form of Ich-1. In mice with reduced Smn levels, the relative amounts of pro- and anti-apoptotic Ich-1 are unaltered indicating correct pre-mRNA splicing, which suggests that SMA is not the result of perturbations of snRNP assembly in motor neurons.[23,38] However, low Smn levels might cause aberrant splicing of motor neuron-specific genes other than *Ich-1*. Further evidence for the notion that splicing defects might not be primary in SMA comes from studies suggesting that the interaction of SMN with Gemins, which is crucial for its function in snRNP biogenesis, might not be as relevant to the SMA pathogenesis as interactions with other factors involved in different processes. For example, mutant SMN proteins lacking the N terminus or exon 7 are unable to bind Sm proteins and certain Gemins but are able to rescue cell lethality in the chicken pre-B cell line DT40 depleted in SMN.[39] Also, the motor abnormalities seen in a *Drosophila* model of SMA can be at least partially rescued by expressing a truncated version of *Drosophila* smn lacking the N terminus, which, as a result of this truncation, is unable to interact with Gemin2.[40,41] Moreover, the general importance of pre-mRNA splicing for cell viability makes it unlikely that reduced accumulation of nuclear snRNPs accounts solely for the motor neuron-specific pathology seen in SMA.

SMN has multiple roles in the nucleus

In addition to its role in snRNP biogenesis and pre-mRNA splicing, SMN is involved in the formation of protein complexes with diverse nuclear functions. SMN has been found in a macromolecular complex with RNA helicase A (RHA), snRNPs and RNA polymerase II indicating a possible role for SMN in transcription.[42] Expression of a dominant negative SMN mutant lacking the N-terminal-most 27 amino acids results in the accumulation of RHA and RNA polymerase II in nuclear aggregates positive for markers of gems and Cajal bodies. As a consequence of this relocalization, gene transcription was found to be inhibited in vivo, possibly due to the reduced nucleoplasmic availability of RNA polymerase II. Other nuclear proteins that have been found to interact with SMN are FUSE-binding protein (FBP),[43] a regulator of transcription and mRNA stability, fibrillarin and GAR1,[44] both of which are components of small nucleolar RNPs (snoRNPs) involved in transcription and processing of ribosomal RNA, and the transcription co-repressor mSin3A.[45] Because of its general role in the assembly of a number of protein complexes involved in transcription regulation and biogenesis of diverse RNPs SMN has also been termed a 'master assembler' molecule.[18]

Axonal and synaptic defects in SMA

SMN is expressed in all tissues examined including heart, central nervous system (CNS), liver, muscle, lung, kidney and pancreas.[7,46] Within the CNS, SMN is widely although not evenly distributed. Here, in addition to the spinal cord where it is found at high levels in the anterior horn motor neurons, SMN has been detected in the brain stem, cerebral cortex, thalamus, basal ganglia, hippocampus and cerebellum.[47,48] Thus, the question arises why primarily spinal cord motor neurons are affected in SMA.[49] According to one hypothesis, motor neurons require particularly high levels of the SMN protein for their development and/or normal function and, therefore, are particularly vulnerable to the reduction of SMN levels.[47,48] Furthermore, SMN might be required for splicing of pre-mRNAs required for motor neuron differentiation and survival.

SMN is present in neurites

An alternative view is that, in addition to its housekeeping role in pre-mRNA splicing and other nuclear processes, SMN might exert motor neuron-specific functions, defects in which

either trigger or at least modulate the SMA disease phenotype.[50] Indeed, there is accumulating evidence that SMN plays a role in neuronal development, in particular in the outgrowth of neurites, which are dendrites conducting impulses towards the neuronal cell body and axons transmitting impulses away from the cell body. SMN is developmentally regulated with the highest levels of expression seen in mammalian embryonic tissues in vivo[38,47,51] and during cell differentiation in vitro.[22,52–54] Similarly, the SMN protein is expressed at higher levels in human fetal tissues compared to the postnatal period.[52] The SMN protein has been detected in vivo in neurites and growth cones of motor neurons in mouse spinal cord,[55] rat spinal cord,[22,48,56] embryonic chick forebrain[22] and in vitro in primary rat cortical neurons transfected with recombinant SMN[57] and in differentiating, neuron-like mouse P19 cells.[50] Fluorescence imaging and electron microscopic analysis confirmed that SMN is associated with axonal microtubules.[22,48,56]

SMA models show axonal and synaptic pathology

From studies on vertebrate and invertebrate models of SMA, evidence has accumulated that SMN exerts roles in axon outgrowth, guidance and the formation of neuromuscular junctions (NMJs). Disruption of the *SMN* homologue in mice results in embryonic lethality and massive cell death prior to implantation of the embryo.[58] To overcome these limitations, Hsieh-Li et al.[59] and Monani et al.[60] generated transgenic mice expressing human *SMN2* in an $Smn^{-/-}$ background. $Smn^{-/-}$*;SMN2* mice containing a low copy number of the *SMN2* transgene show severe motor defects and die shortly after birth. Primary motor neurons isolated from the lumbar region of the spinal cord of $Smn^{-/-}$*;SMN2* mice show the same survival characteristics as motor neurons from $Smn^{+/+}$*;SMN2* mice when cultured in the absence or presence of neurotrophic factors (BDNF and CNTF).[61] In contrast, $Smn^{-/-}$*;SMN2* motor neurons have significantly shorter axons than controls due to disruption of actin localization in motor axons and growth cones. Taken together, these data seem to indicate that, in this SMA cell culture model, low levels of SMN affect axon outgrowth but not motor neuron survival. When interpreting these results, it has to be considered, however, that even though SMN reduction might not affect motor neuron survival in vitro, it is likely that defects in growth cone movement and failure to establish contact with the target muscle are critical to age-dependent programmed cell death in vivo. $Smn^{-/-}$*;SMN2* mice carrying a high-copy *SMN2* transgene do not display any motor abnormalities indicating that enhanced *SMN2* gene expression can generate sufficient amounts of functional SMN to prevent motor neuron degeneration.[60] This finding is of relevance for future therapeutic strategies as compounds have been identified that upregulate *SMN2* transcription.[62–64] Also of possible significance in the therapeutic context is the new finding that multiple injections of SMN-expressing lentivector into different muscles delay the onset of motor abnormalities and extend the life span in a mouse model of SMA.[65] SMN levels in motor neurons were found to be restored due to retrograde axonal transport of the SMN-containing viral vector. Thus, SMN replacement through gene therapy prior to the onset of motor symptoms may provide an additional treatment option for human SMA.

Using a different approach to circumvent the embryonic lethality associated with homozygous loss of *Smn*, Frugier et al. created transgenic mice harbouring an *Smn* exon 7 deletion confined to spinal cord motor neurons.[66] These "neuronal" *Smn* mutant mice exhibit only a mild loss of motor neuron cell bodies but show a significant reduction in myelinated axons in ventral roots of the spinal cord indicating that proximal axon degeneration is an early neuropathological hallmark in this SMA model and precedes motor neuron cell death.[67] Consistent with this finding, terminal axons are significantly reduced in number and NMJ postsynaptic folds often lack the apposed terminal axon indicating muscle denervation. Furthermore, the synaptic terminals of NMJs in mutant mice show severe defects including lack of axonal sprouting, reduced postsynaptic areas and aberrant accumulation of phosphorylated neurofilaments. The authors suggest that a 'dying-back' axonopathy involving destabilization of cytoskeletal components results in motor neuron dysfunction in this SMA model. Consistent with the cytoskeletal abnormalities seen in these mutant mice, defects in neurofilament organization have also been observed in patients with Type I SMA.[68,69] Furthermore, mutations in or overexpression of neurofilament subunits can induce selective motor neuron degeneration resembling motor neuron disease, suggesting a possible primary role for cytoskeleton abnormalities.[70] However, the "neuronal" *Smn* mouse mutant may not faithfully replicate human SMA because, in this model, Smn is inactivated completely and selectively in motor neurons as opposed to an incomplete but global loss of SMN in the human disease. For example, there is no pathological evidence for axonal demyelination in human SMA, whereas the "neuronal" *Smn* mutant mice exhibit strong myelination defects.

A dysfunctional NMJ has also been observed in a fly model of SMA. Chan et al.[41] identified a *Drosphila smn* mutant that, unlike mice lacking *Smn*, progresses through embryogenesis due to maternal contribution of wild-type *smn* mRNA but dies as late larvae. Mutant larvae display locomotory defects that can be rescued by expressing full-length Smn protein but not a truncated form lacking the C terminus. The same authors showed that NMJs of *smn* mutant *Drosophila* muscle cells exhibit significantly reduced excitatory postsynaptic currents (EPSCs), have an abnormal architecture and show aberrant postsynaptic clustering of glutamate receptors. An axonal pathology was observed in a zebrafish model of SMA in which *smn* is knocked down using antisense morpholino technology.[71] Embryos injected with Smn morpholinos contain motor

neurons with truncated and abnormally branched axons. Both ventrally and dorsally projecting motor axons are affected and the severity of the defects can be enhanced by injecting Smn morpholinos at higher concentrations resulting in increased knockdown effects. These axonal defects are not caused by motor neuron cell death as was revealed by TUNEL staining, which is in accordance with mouse models of SMA in which motor neuron death is a relatively late manifestation of the disease.[60,66] In the zebrafish model of SMA, neither interneurons nor sensory neurons are affected by low levels of Smn indicating that, in this system, as in SMA, motor neurons show a unique sensitivity for reducs amounts of Smn protein. Furthermore, muscle development proceeds normally in zebrafish embryos injected with Smn morpholinos and motor axons produce normal postsynaptic acetylcholine receptor (AChR) clustering suggesting that motor axons are still capable of innervating muscle fibers but show defects in pathfinding.

Despite the fact that these animal models of SMA appear to differ somewhat with respect to their precise pathological hallmarks and thus may not fully mimic the human disease, they do nevertheless point to possible roles for SMN in neuronal processes.

Even though the majority of studies on SMA models investigate the functional role of SMN in neurons alone, there are some reports for a primary muscle defect. For example, the motor phenotype seen in the *Drosophila smn* mutant can be partially rescued by expressing the *smn* gene in muscle tissue.[41] Furthermore, targeted deletion of murine *Smn* exon 7 in skeletal muscle results in a dystrophic phenotype with disruption of the dystrophin complex indicating a contribution of muscle SMN loss to the human disease.[72] Neuromuscular junction architecture and motor neuron morphology and number is normal in this "muscular" *Smn* mutant indicating that loss of muscle Smn does not account for motor neuron degeneration in this SMA model. The importance of muscle SMN reduction for SMA, however, has recently been questioned in a study demonstrating that mice expressing human *SMN2* and an *SMNΔ7* transgene lacking exon 7 in an $Smn^{-/-}$ background exhibit motor neuron degeneration but do not show a dystrophic phenotype.[73] Thus, low levels of functional SMN, as are present in SMA, seem to be sufficient to prevent muscle dystrophy.

A novel, axon-specific SMN protein complex?

It has been suggested that SMN functions in neuronal development by associating with distinct molecular factors in the cytoplasm and in neurites.[55] Thus, by forming different macromolecular protein complexes in particular subcellular regions, the range of functions of SMN may be broader than previously envisaged involving cellular processes other than snRNP biogenesis and pre-mRNA splicing.

In an attempt to identify novel SMN interactors, Rossoll et al.[74] performed a yeast-two-hybrid screen using murine Smn as bait. Among interacting proteins were the highly related hnRNP-R and hnRNP-Q, both of which contain an RNA recognition motif (RRM) and an arginine-glycine-rich C-terminal region. In co-immunoprecipitation experiments, hnRNP-R and hnRNP-Q were found to bind to wild-type murine Smn but not to isoforms containing SMA-causing human mutations including E143K, Y272C and deletion of exon 7. Similar to Smn both hnRNP-R and hnRNP-Q are ubiquitously expressed and developmentally regulated with the highest levels of expression observed during late embryogenesis. Subsequent immunohistochemical analysis revealed that hnRNP-R is predominantly present in the cytoplasm and axons of motor neurons, where it co-localizes with Smn, but not in nuclear gems. These data suggest that hnRNP-R associates with Smn to mediate cellular processes other than pre-mRNA splicing.

In addition to interactions with hnRNP-R and Q, SMN has also been shown to interact with profilin II, an actin-binding protein, via its highly conserved polyproline motif.[75] Profilin II is highly expressed in mouse brain and spinal cord, where it locates predominantly to anterior horn neurons.[75,76] Profilin II is involved in actin polymerization and has been implicated in synaptic recycling and axon outgrowth.[76,77]

SMN and axonal transport

Using cultured cells, Rossoll et al.[61] investigated the function of the Smn interaction with hnRNP-R. In PC12 cells, Smn co-localizes with hnRNP-R in cell bodies and neurite-like processes. In contrast, hnRNP-R lacking the Smn interaction domain localizes primarily to the nucleus indicating that hnRNP-R is targeted to cell processes by Smn. In accordance with these data, the authors also demonstrated that hnRNP-R is present only at significantly reduced levels in neurites and growth cones of motor neurons from $Smn^{-/-}$*;SMN2* mice compared to $Smn^{+/+}$*;SMN2* motor neurons. By using a cell culture-based method, the authors showed that the interaction between Smn and hnRNP-R is functionally relevant. Upregulation of either Smn or hnRNP-R in PC12 cells results in enhanced outgrowth of neurites, which is suppressed by hnRNP-R lacking the Smn interaction domain. By expressing truncated proteins, the authors also showed that this positive effect on neurite outgrowth depends on functional Smn as well as on the RNA-binding domain of hnRNP-R. Accordingly, *Smn* mutant motor neurons show reduced growth cone size. To further investigate axon-specific functions of Smn, the authors identified actin as a possible downstream mediator of the Smn–hnRNP–R complex. Actin is a major component of outgrowing axons and transport of its mRNA into distal axons and growth cones is stimulated by neurotrophin treatment.[78–81] In developing neurons, reduction of β-actin mRNA and protein results in impaired growth cone motility.[80] Immunohistochemical analysis revealed that actin is present

at significantly lower levels in neurites and growth cones of motor neurons isolated from *Smn*$^{-/-}$*;SMN2* embryos compared to controls. By using biotinylated actin-specific antisense probes, Rossoll and colleagues showed that the distal β-actin mRNA translocation is dependent on Smn as well as functional hnRNP-R (containing the RNA recognition domain and the Smn interaction domain). Finally, using immunoprecipitation techniques, the authors showed that hnRNP-R binds to the 3′ UTR of β-actin mRNA both in vitro and in vivo. The authors suggest that the precise role of the axonal Smn–hnRNP-R complex in actin accumulation involves transport and/or translational regulation of β-actin mRNA. Ribosome-like particles have been detected in axons and growth cones allowing for the possibility that β-actin mRNA is translated locally into protein.[23] Due to its role in β-actin mRNA trafficking, SMN might therefore indirectly regulate the amount of 'local' actin used for growth cone behaviour and synapse function. Thus, reduction of SMN levels might result in a decreased availability of actin monomers for polymerization leading to defects in actin-dependent processes such as motor growth cone movement, neurite outgrowth, presynaptic differentiation and synaptic vesicle trafficking and release.[61]

Localization and transport of β-actin mRNA in neurites has previously been shown to be regulated by the zipcode-binding proteins (ZBPs) 1 and 2 which bind to the zipcode sequence located in the 3′ UTR of β-actin mRNA.[80,82] An RNP complex containing ZBP 1 and β-actin mRNA is actively transported into neuronal processes and growth cones in response to neurotrophin-3.[80] Disruption of this RNP complex by antisense oligonucleotides to the zipcode sequence prevents β-actin mRNA translocation and produces aberrant growth cone behaviour. Similarly, overexpression of a dominant negative ZBP 2 truncated protein significantly reduces β-actin mRNA localization to processes and growth cones in developing chick neurons.[82] It remains to be determined whether the SMN–hnRNP-R/Q complex and the ZBPs are components of the same localization pathway.

Consistent with the results obtained by Rossoll and colleagues[61] and by studies showing co-localization of SMN with axonal microtubules,[48,56] Zhang et al.[22] demonstrated that cytoplasmic SMN exhibits dynamic movements in neuronal processes. Using live cell imaging, the authors showed that SMN tagged with enhanced green fluorescent protein form granules that move bidirectionally in neuronal processes. The authors distinguished between small SMN granules, which exhibit persistent movements over long distances, and large granules, which move predominantly in an oscillatory fashion. These movements are dependent on the cytoskeletal filament system with long-distance movements relying primarily on microtubules and the oscillatory behaviour depending on actin filaments. The speed of movement was found to be consistent with fast axonal transport, which is mediated by motor proteins such as kinesin and dynein family proteins.[83]

Further support for the notion that defects in axonal transport might represent a pathophysiological event in SMA comes from the intriguing finding that mutations in microtubule-dependent motor proteins can recapitulate the motor neuron-specific pathology characteristic for SMA. With their extended axonal processes, motor neurons critically rely on active anterograde transport to deliver cellular material synthesized in the cell body into distal regions of the axon. Conversely, motor neuron survival depends on the availability of neurotrophic factors from the cell surrounding obtained through internalization followed by retrograde transport from the periphery of the cell to the soma.[84] Furthermore, during development, motor neurons require efficient transport of cytoskeletal elements for diverse processes including growth cone migration, axon branching and synapse formation and stability. Thus, given the large demand of motor neurons for axonal transport, it has been hypothesized that these cells are particularly vulnerable to disruption of the microtubule-dependent transport system.[24] Indeed, work by LaMonte et al.[85] has demonstrated that, in mice, inhibition of retrograde axonal transport through targeted disruption of the dynein–dynactin complex is sufficient to induce motor neuron degeneration accompanied by skeletal muscle atrophy. Importantly, the study by Hafezparast et al.[86] suggests that motor neurons are particularly susceptible to disruption of axonal transport. Mice bearing missense point mutations in the dynein heavy chain show a selective loss of spinal cord anterior horn cells, accompanied by a marked decrease in fast retrograde transport. Defects in axonal transport have been associated with a number of diseases affecting upper and lower motor neurons including amyotrophic lateral sclerosis,[87] Charcot-Marie-Tooth disease type 2A,[88] Huntington's disease,[89] spinal bulbar muscular atrophy,[90] hereditary spastic paraplegia[91] and motor neuron disease.[92] The finding by Rossoll et al.[61] suggesting that transport of the β-actin signal along the axon is compromised in motor neurons lacking SMN strongly indicates that, also in SMA axonal transport, defects might be at the root of the disease (Fig. 3).

It is of interest that abnormal neurofilament accumulation has been found to accompany defects in axonal transport in a number of studies.[85–87] Thus, it is conceivable that the neurofilament aggregates seen in mice with neuronal SMN exon 7 deletion[67] reflect a general slowing of axonal transport in this SMA model.

Cytoplasmic targeting of SMN is essential for neurite outgrowth

The identification of novel SMN-binding partners in neuronal processes has led to the suggestion that the SMA-causing

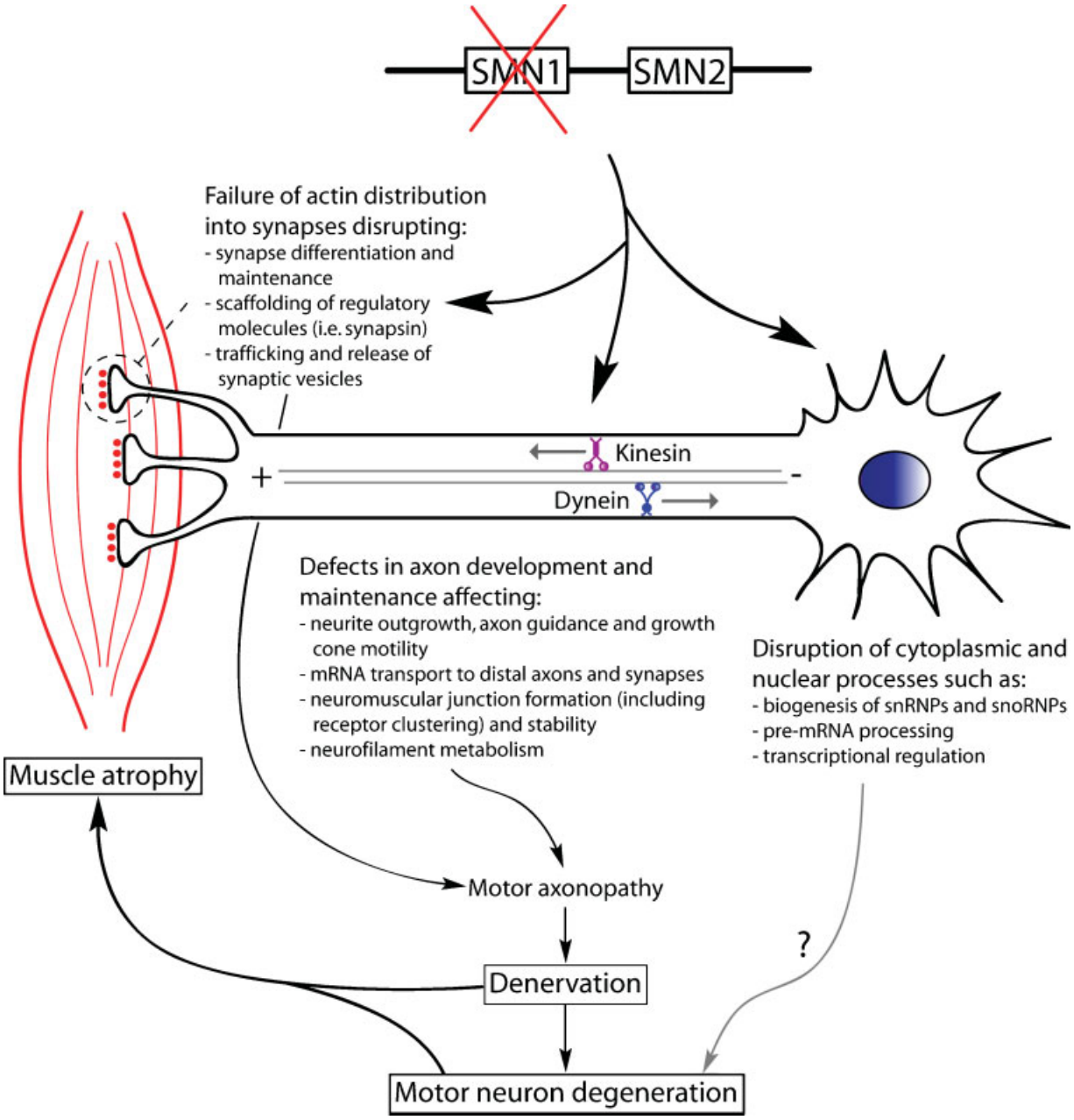

Figure 3. Schematic representation of motor neuron-associated pathogenic events occurring in SMA as a result of reduced SMN activity. The majority of *SMN2* transcripts lack exon 7 generating truncated, non-functional protein, which is unable to compensate for the loss of *SMN1*. The SMN protein has been implicated in a number of nuclear and cytoplasmic processes including spliceosome formation and gene transcription, disruptions of which may contribute to the SMA pathogenesis. Furthermore, loss of muscle SMN may contribute to the disease as deletion of murine *Smn* exon 7 confined to skeletal muscle produces a dystrophic phenotype.[72] A number of recent studies suggest that SMN exerts functions in the neuronal periphery, i.e. neurites and growth cones, defects in which may induce motor neuron cell death. Several neurite-specific SMN-interacting proteins have been identified indicating the existence of a distinct SMN complex in neuronal processes. The dynamic localization of SMN in axons may be important for the transport of diverse ribonucleoproteins to distal regions during axon outgrowth and maintenance. Consistent with this idea, cultured neurons overexpressing truncated SMN lacking exon 7 have significantly shorter neurites implying a role for SMN in motor neuron development.[22] Furthermore, disruption of actin localization to growth cones and synapses in motor neurons with reduced SMN levels may affect the establishment of appropriate synaptic connections during development and disturb presynaptic processes such as neurotransmitter release.[104,105] Defects in these peripheral processes may play key roles in eliciting motor neuron degeneration in SMA leading to neurogenic atrophy of skeletal muscle.

mutations of SMN induce, or at least contribute to, the motor neuron-specific pathology by disrupting interactions between SMN and axonal proteins. Furthermore, changes in the subcellular localization of SMN as a result of such mutations might reduce its cytoplasmic availability and, thus, indirectly affect its axon-specific functions. Dodds et al.[57] showed that SMN expressed in primary rat cortical neurons preferentially localizes as aggregates throughout the cytoplasm including neurites, whereas the SMNΔ7 truncated protein lacking exon 7 is primarily targeted to the nucleus. The authors suggest that exon 7 encodes a nuclear export signal that regulates intracellular trafficking of the SMN protein. Consistent with these data, Zhang et al.[22] observed that truncated SMNΔ7 localizes exclusively to the nucleus in transfected chick forebrain neurons but is absent from cytoplasm and processes. In contrast, deletion of exon 5 has no effect on the subcellular distribution of SMN. Insertion of exon 7 into the C terminus of D-box-binding factor 1 (DBF1), a nuclear protein, is sufficient for its re-distribution to the cytoplasm confirming that exon 7 encodes a cytoplasmic targeting signal. By generating DBF1 and SMNΔ7 reporter constructs containing only subregions of exon 7, Zhang and colleagues identified the amino acid sequence QNQKE to be sufficient for cytoplasmic targeting. Similar to previous reports, these authors observed that neurons overexpressing SMNΔ7 show a 25% reduction in neurite length confirming that the cytoplasmic localization of SMN is functionally relevant. Interestingly, the neurite length can be restored by tagging SMNΔ7 with the cytoplasmic targeting sequence of GAP-43 indicating that neurite growth depends on SMN being present in the cytoplasm and in neuronal processes.

Is SMA developmental or degenerative in origin?

Since the identification of *SMN* as the SMA-determining gene, considerable progress has been made in the biochemical characterization of the SMN protein as well as in the generation of models of the disease. SMN has been implicated in both nuclear and cytoplasmic processes related to mRNA processing, i.e. gene transcription and biogenesis of spliceosomal components, and, thus, provides an example for multifunctional regulatory proteins acting in different cellular compartments to control RNA metabolism.[93] It is not immediately obvious, however, how these functions of SMN relate to the disease pathology of SMA characterized by selective loss of lower motor neurons. Cell culture and animal models point towards the possibility that SMN exerts functions in the neuronal periphery. Defects in these functions might affect neurodevelopmental processes such as outgrowth of motor axons and innervation of target muscle, axon arborization, presynaptic differentiation, postsynaptic receptor clustering driven by neurotransmitter signalling, organization of the cytoskeleton in axons and at the NMJ, and axonal transport of structural and regulatory components to the synapse. The mechanism underlying these developmental, neurite-specific functions of SMN might involve the assembly of mRNAs required for neurite development and stability into mRNPs and their translocation to distal axons and NMJs.

SMA represents a spectrum of different severities, largely due to the dosage effect of *SMN2*, which has been phenocopied in mouse models of the disease. Based on findings from animal models of SMA, it could thus be argued that the extent to which motor neurons proceed through development to establish functional connections depends on the amount of functional SMN available. In the severe SMA forms, in which motor neurons show defects in axon outgrowth and fail to connect properly to their target muscle causing muscle atrophy, there appears to be a relatively strong developmental defect. In contrast, in the milder forms of SMA, where motor neurons grow towards and innervate their target muscles but, nevertheless, show abnormalities, for example in neurotransmission, such defects are initially subtle, yet over time become sufficient to induce late-onset denervation, motor neuron degeneration and muscle atrophy.

To test the hypothesis that, in SMA, developmental defects precede motor neuron degeneration and that the extent to which motor neurons proceed through normal development depends on the availability of functional SMN, different aspects of the neuromuscular system should be monitored over time in animal models of the disease. This hypothesis predicts that, in SMA, gross morphological defects of motor neuron cell bodies might only be a late manifestation of degenerative events occurring much earlier on a molecular level in the neuronal periphery. Electrophysiological approaches, for example, could help to detect early changes in efficiency of synaptic transmission at the NMJ in SMA models. Morphological changes of motor neuron terminals and postsynaptic structures could also be investigated before the onset of visible motor symptoms as such early structural abnormalities are likely to indicate defects in motor neuron function. Furthermore, biochemical characterization of the neurite-specific SMN complex might help to better understand its role in axonal transport during axonogenesis.

The diversity of the pathological hallmarks observed in vertebrate and invertebrate SMA models strongly suggests that the severity of the corresponding phenotype depends on the extent of SMN downregulation achieved, thus making a direct comparison difficult. An important experimental challenge will be to further optimize current techniques for gene downregulation in order to be able to adjust SMN to a level that allows embryogenesis to occur but produces a neuromuscular phenotype amenable for study.

The recent observations on axon-specific functions of SMN are important from a neuropathological perspective for future investigations into the causes of motor neuron cell death seen in SMA. Motor neuron degeneration might start at the peri-

phery, i.e. in synapses and axons, and manifest itself as slowing of axonal transport and cytoskeletal abnormalities leading to motor neuron cell death as a secondary effect (Fig. 3). These cellular changes might occur long before the onset of clinical symptoms and, therefore, provide a target for therapeutic strategies aimed at delaying the onset of motor neuron degeneration.

Acknowledgments

The authors thank Kay E. Davies, Kevin Talbot, Nicholas Owen, Marcel van den Heuvel and Sarah L. Boddy for helpful discussions during the preparation of the manuscript.

References

1. Zerres K, Davies KE. 1999. 59th ENMC International Workshop: Spinal Muscular Atrophies: recent progress and revised diagnostic criteria 17–19 April 1998, Soestduinen, The Netherlands. Neuromuscul Disord 9:272–278.
2. Dubowitz V. 1999. Very severe spinal muscular atrophy (SMA type 0): an expanding clinical phenotype. Eur J Paediatr Neurol 3:49–51.
3. MacLeod MJ, Taylor JE, Lunt PW, Mathew CG, Robb SA. 1999. Prenatal onset spinal muscular atrophy. Eur J Paediatr Neurol 3:65–72.
4. Pearn JH, Hudgson P, Walton JN. 1978. A clinical and genetic study of spinal muscular atrophy of adult onset: the autosomal recessive form as a discrete disease entity. Brain 101:591–606.
5. Brzustowicz LM, Lehner T, Castilla LH, Penchaszadeh GK, Wilhelmsen KC, et al. 1990. Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3. Nature 344:540–541.
6. Brzustowicz LM, Kleyn PW, Boyce FM, Lien LL, Monaco AP, et al. 1992. Fine-mapping of the spinal muscular atrophy locus to a region flanked by MAP1B and D5S6. Genomics 13:991–998.
7. Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, et al. 1995. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80:155–165.
8. Schmutz J, Martin J, Terry A, Couronne O, Grimwood J, et al. 2004. The DNA sequence and comparative analysis of human chromosome 5. Nature 431:268–274.
9. Kashima T, Manley JL. 2003. A negative element in *SMN2* exon 7 inhibits splicing in spinal muscular atrophy. Nat Genet 34:460–463.
10. Lorson CL, Androphy EJ. 2000. An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene *SMN*. Hum Mol Genet 9:259–265.
11. Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, Davies KE. 1995. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. Hum Mol Genet 4:631–634.
12. Burghes AH. 1997. When is a deletion not a deletion? When it is converted. Am J Hum Genet 61:9–15.
13. Yamashita M, Nishio H, Harada Y, Matsuo M, Yamamoto T. 2004. Significant increase in the number of the *SMN2* gene copies in an adult-onset Type III spinal muscular atrophy patient with homozygous deletion of the *NAIP* gene. Eur Neurol 52:101–106.
14. Frugier T, Nicole S, Cifuentes-Diaz C, Melki J. 2002. The molecular bases of spinal muscular atrophy. Curr Opin Genet Dev 12:294–298.
15. Brahe C, Servidei S, Zappata S, Ricci E, Tonali P, Neri G. 1995. Genetic homogeneity between childhood-onset and adult-onset autosomal recessive spinal muscular atrophy. Lancet 346:741–742.
16. Clermont O, Burlet P, Lefebvre S, Burglen L, Munnich A, Melki J. 1995. *SMN* gene deletions in adult-onset spinal muscular atrophy. Lancet 346: 1712–1713.
17. Zerres K, Rudnik-Schoneborn S, Forkert R, Wirth B. 1995. Genetic basis of adult-onset spinal muscular atrophy. Lancet 346:1162.
18. Terns MP, Terns RM. 2001. Macromolecular complexes: SMN–the master assembler. Curr Biol 11:R862–864.
19. Paushkin S, Gubitz AK, Massenet S, Dreyfuss G. 2002. The SMN complex, an assemblyosome of ribonucleoproteins. Curr Opin Cell Biol 14:305–312.
20. Meister G, Eggert C, Fischer U. 2002. SMN-mediated assembly of RNPs: a complex story. Trends Cell Biol 12:472–478.
21. Yong J, Wan L, Dreyfuss G. 2004. Why do cells need an assembly machine for RNA-protein complexes? Trends Cell Biol 14:226–232.
22. Zhang HL, Pan F, Hong D, Shenoy SM, Singer RH, Bassell GJ. 2003. Active transport of the survival motor neuron protein and the role of exon-7 in cytoplasmic localization. J Neurosci 23:6627–6637.
23. Jablonka S, Wiese S, Sendtner M. 2004. Axonal defects in mouse models of motoneuron disease. J Neurobiol 58:272–286.
24. Holzbaur EL. 2004. Motor neurons rely on motor proteins. Trends Cell Biol 14:233–240.
25. Hannus S, Buhler D, Romano M, Seraphin B, Fischer U. 2000. The *Schizosaccharomyces pombe* protein Yab8p and a novel factor, Yip1p, share structural and functional similarity with the spinal muscular atrophy-associated proteins SMN and SIP1. Hum Mol Genet 9:663–674.
26. Miguel-Aliaga I, Culetto E, Walker DS, Baylis HA, Sattelle DB, Davies KE. 1999. The *Caenorhabditis elegans* orthologue of the human gene responsible for spinal muscular atrophy is a maternal product critical for germline maturation and embryonic viability. Hum Mol Genet 8: 2133–2143.
27. Pellizzoni L, Baccon J, Rappsilber J, Mann M, Dreyfuss G. 2002. Purification of native survival of motor neurons complexes and identification of Gemin6 as a novel component. J Biol Chem 277:7540–7545.
28. Baccon J, Pellizzoni L, Rappsilber J, Mann M, Dreyfuss G. 2002. Identification and characterization of Gemin7, a novel component of the survival of motor neuron complex. J Biol Chem 277:31957–31962.
29. Liu Q, Dreyfuss G. 1996. A novel nuclear structure containing the survival of motor neurons protein. Embo J 15:3555–3565.
30. Narayanan U, Achsel T, Luhrmann R, Matera AG. 2004. Coupled in vitro import of U snRNPs and SMN, the spinal muscular atrophy protein. Mol Cell 16:223–234.
31. Pellizzoni L, Yong J, Dreyfuss G. 2002. Essential role for the SMN complex in the specificity of snRNP assembly. Science 298:1775–1779.
32. Yong J, Golembe TJ, Battle DJ, Pellizzoni L, Dreyfuss G. 2004. snRNAs contain specific SMN-binding domains that are essential for snRNP assembly. Mol Cell Biol 24:2747–2756.
33. Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G. 1998. A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell 95:615–624.
34. Jablonka S, Holtmann B, Meister G, Bandilla M, Rossoll W, et al. 2002. Gene targeting of *Gemin2* in mice reveals a correlation between defects in the biogenesis of U snRNPs and motoneuron cell death. Proc Natl Acad Sci USA 99:10126–10131.
35. Buhler D, Raker V, Luhrmann R, Fischer U. 1999. Essential role for the tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. Hum Mol Genet 8:2351–2357.
36. Pellizzoni L, Charroux B, Dreyfuss G. 1999. SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins. Proc Natl Acad Sci USA 96:11167–11172.
37. Wirth B. 2000. An update of the mutation spectrum of the survival motor neuron gene (*SMN1*) in autosomal recessive spinal muscular atrophy (SMA). Hum Mutat 15:228–237.
38. Jablonka S, Schrank B, Kralewski M, Rossoll W, Sendtner M. 2000. Reduced survival motor neuron (*Smn*) gene dose in mice leads to motor neuron degeneration: an animal model for spinal muscular atrophy type III. Hum Mol Genet 9:341–346.
39. Wang J, Dreyfuss G. 2001. Characterization of functional domains of the SMN protein in vivo. J Biol Chem 276:45387–45393.
40. Liu Q, Fischer U, Wang F, Dreyfuss G. 1997. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell 90:1013–1021.
41. Chan YB, Miguel-Aliaga I, Franks C, Thomas N, Trulzsch B, et al. 2003. Neuromuscular defects in a *Drosophila* survival motor neuron gene mutant. Hum Mol Genet 12:1367–1376.
42. Pellizzoni L, Charroux B, Rappsilber J, Mann M, Dreyfuss G. 2001. A functional interaction between the survival motor neuron complex and RNA polymerase II. J Cell Biol 152:75–85.

43. Williams BY, Hamilton SL, Sarkar HK. 2000. The survival motor neuron protein interacts with the transactivator FUSE binding protein from human fetal brain. FEBS Lett 470:207–210.
44. Pellizzoni L, Baccon J, Charroux B, Dreyfuss G. 2001. The survival of motor neurons (SMN) protein interacts with the snoRNP proteins fibrillarin and GAR1. Curr Biol 11:1079–1088.
45. Zou J, Barahmand-pour F, Blackburn ML, Matsui Y, Chansky HA, Yang L. 2004. Survival motor neuron (SMN) protein interacts with transcription corepressor mSin3A. J Biol Chem 279:14922–14928.
46. Novelli G, Calza L, Amicucci P, Giardino L, Pozza M, et al. 1997. Expression study of survival motor neuron gene in human fetal tissues. Biochem Mol Med 61:102–106.
47. Battaglia G, Princivalle A, Forti F, Lizier C, Zeviani M. 1997. Expression of the *SMN* gene, the spinal muscular atrophy determining gene, in the mammalian central nervous system. Hum Mol Genet 6:1961–1971.
48. Bechade C, Rostaing P, Cisterni C, Kalisch R, La Bella V, Pettmann B, Triller A. 1999. Subcellular distribution of survival motor neuron (SMN) protein: possible involvement in nucleocytoplasmic and dendritic transport. Eur J Neurosci 11:293–304.
49. Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O, et al. 1997. Correlation between severity and SMN protein level in spinal muscular atrophy. Nat Genet 16:265–269.
50. Fan L, Simard LR. 2002. Survival motor neuron (SMN) protein: role in neurite outgrowth and neuromuscular maturation during neuronal differentiation and development. Hum Mol Genet 11:1605–1614.
51. La Bella V, Cisterni C, Salaun D, Pettmann B. 1998. Survival motor neuron (SMN) protein in rat is expressed as different molecular forms and is developmentally regulated. Eur J Neurosci 10:2913–2923.
52. Burlet P, Huber C, Bertrandy S, Ludosky MA, Zwaenepoel I, et al. 1998. The distribution of SMN protein complex in human fetal tissues and its alteration in spinal muscular atrophy. Hum Mol Genet 7:1927–1933.
53. Germain-Desprez D, Brun T, Rochette C, Semionov A, Rouget R, Simard LR. 2001. The *SMN* genes are subject to transcriptional regulation during cellular differentiation. Gene 279:109–117.
54. Rouget R, Vigneault F, Codio C, Rochette C, Paradis I, et al. 2005. Characterization of the survival motor neuron (*SMN*) promoter provides evidence for complex combinatorial regulation in undifferentiated and differentiated P19 cells. Biochem J 385:433–443.
55. Jablonka S, Bandilla M, Wiese S, Buhler D, Wirth B, et al. 2001. Co-regulation of survival of motor neuron (SMN) protein and its interactor SIP1 during development and in spinal muscular atrophy. Hum Mol Genet 10:497–505.
56. Pagliardini S, Giavazzi A, Setola V, Lizier C, Di Luca M, et al. 2000. Subcellular localization and axonal transport of the survival motor neuron (SMN) protein in the developing rat spinal cord. Hum Mol Genet 9:47–56.
57. Dodds E, Dunckley MG, Roberts RG, Muntoni F, Shaw CE. 2001. Overexpressed human survival motor neurone isoforms, *SMNDeltaexon7* and *SMN+exon7*, both form intranuclear gems but differ in cytoplasmic distribution. FEBS Lett 495:31–38.
58. Schrank B, Gotz R, Gunnersen JM, Ure JM, Toyka KV, et al. 1997. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci USA 94:9920–9925.
59. Hsieh-Li HM, Chang JG, Jong YJ, Wu MH, Wang NM, Tsai CH, Li H. 2000. A mouse model for spinal muscular atrophy. Nat Genet 24: 66–70.
60. Monani UR, Sendtner M, Coovert DD, Parsons DW, Andreassi C, et al. 2000. The human centromeric survival motor neuron gene (*SMN2*) rescues embryonic lethality in Smn(−/−) mice and results in a mouse with spinal muscular atrophy. Hum Mol Genet 9:333–339.
61. Rossoll W, Jablonka S, Andreassi C, Kroning AK, Karle K, et al. 2003. Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. J Cell Biol 163:801–812.
62. Andreassi C, Patrizi AL, Monani UR, Burghes AH, Brahe C, Eboli ML. 2002. Expression of the survival of motor neuron (*SMN*) gene in primary neurons and increase in SMN levels by activation of the N-methyl-D-aspartate glutamate receptor. Neurogenetics 4:29–36.
63. Andreassi C, Angelozzi C, Tiziano FD, Vitali T, De Vincenzi E, et al. 2004. Phenylbutyrate increases *SMN* expression in vitro: relevance for treatment of spinal muscular atrophy. Eur J Hum Genet 12:59–65.
64. Brichta L, Hofmann Y, Hahnen E, Siebzehnrubl FA, Raschke H, et al. 2003. Valproic acid increases the SMN2 protein level: a well-known drug as a potential therapy for spinal muscular atrophy. Hum Mol Genet 12:2481–2489.
65. Azzouz M, Le T, Ralph GS, Walmsley L, Monani UR, et al. 2004. Lentivector-mediated *SMN* replacement in a mouse model of spinal muscular atrophy. J Clin Invest 114:1726–1731.
66. Frugier T, Tiziano FD, Cifuentes-Diaz C, Miniou P, Roblot N, et al. 2000. Nuclear targeting defect of SMN lacking the C-terminus in a mouse model of spinal muscular atrophy. Hum Mol Genet 9:849–858.
67. Cifuentes-Diaz C, Nicole S, Velasco ME, Borra-Cebrian C, Panozzo C, et al. 2002. Neurofilament accumulation at the motor endplate and lack of axonal sprouting in a spinal muscular atrophy mouse model. Hum Mol Genet 11:1439–1447.
68. Lippa CF, Smith TW. 1988. Chromatolytic neurons in Werdnig-Hoffmann disease contain phosphorylated neurofilaments. Acta Neuropathol (Berl) 77:91–94.
69. Murayama S, Bouldin TW, Suzuki K. 1991. Immunocytochemical and ultrastructural studies of Werdnig-Hoffmann disease. Acta Neuropathol (Berl) 81:408–417.
70. Perrone Capano C, Pernas-Alonso R, di Porzio U. 2001. Neurofilament homeostasis and motoneurone degeneration. Bioessays 23:24–33.
71. McWhorter ML, Monani UR, Burghes AH, Beattie CE. 2003. Knockdown of the survival motor neuron (Smn) protein in zebrafish causes defects in motor axon outgrowth and pathfinding. J Cell Biol 162:919–931.
72. Cifuentes-Diaz C, Frugier T, Tiziano FD, Lacene E, Roblot N, et al. 2001. Deletion of murine *SMN* exon 7 directed to skeletal muscle leads to severe muscular dystrophy. J Cell Biol 152:1107–1114.
73. Le TT, Pham LT, Butchbach ME, Zhang HL, Monani UR, et al. 2005. *SMNΔ7*, the major product of the centromeric survival motor neuron (*SMN2*) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. Hum Mol Genet 14:845–857.
74. Rossoll W, Kroning AK, Ohndorf UM, Steegborn C, Jablonka S, Sendtner M. 2002. Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and gry-rbp/hnRNP-Q: a role for Smn in RNA processing in motor axons? Hum Mol Genet 11:93–105.
75. Giesemann T, Rathke-Hartlieb S, Rothkegel M, Bartsch JW, Buchmeier S, et al. 1999. A role for polyproline motifs in the spinal muscular atrophy protein SMN. Profilins bind to and colocalize with smn in nuclear gems. J Biol Chem 274:37908–37914.
76. Witke W, Podtelejnikov AV, Di Nardo A, Sutherland JD, Gurniak CB, et al. 1998. In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly. Embo J 17:967–976.
77. Wills Z, Marr L, Zinn K, Goodman CS, Van Vactor D. 1999. Profilin and the Abl tyrosine kinase are required for motor axon outgrowth in the Drosophila embryo. Neuron 22:291–299.
78. Bassell GJ, Zhang H, Byrd AL, Femino AM, Singer RH, et al. 1998. Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. J Neurosci 18:251–265.
79. Zhang HL, Singer RH, Bassell GJ. 1999. Neurotrophin regulation of beta-actin mRNA and protein localization within growth cones. J Cell Biol 147:59–70.
80. Zhang HL, Eom T, Oleynikov Y, Shenoy SM, Liebelt DA, et al. 2001. Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. Neuron 31:261–275.
81. Willis D, Li KW, Zheng JQ, Chang JH, Smit A, et al. 2005. Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. J Neurosci 25:778–791.
82. Gu W, Pan F, Zhang H, Bassell GJ, Singer RH. 2002. A predominantly nuclear protein affecting cytoplasmic localization of beta-actin mRNA in fibroblasts and neurons. J Cell Biol 156:41–51.
83. Hirokawa N. 1998. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279:519–526.
84. Chao MV. 2003. Retrograde transport redux. Neuron 39:1–2.

85. LaMonte BH, Wallace KE, Holloway BA, Shelly SS, Ascano J, et al. 2002. Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration. Neuron 34:715–727.
86. Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A, et al. 2003. Mutations in dynein link motor neuron degeneration to defects in retrograde transport. Science 300:808–812.
87. Williamson TL, Cleveland DW. 1999. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat Neurosci 2:50–56.
88. Zhao C, Takita J, Tanaka Y, Setou M, Nakagawa T, et al. 2001. Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. Cell 105:587–597.
89. Gunawardena S, Her LS, Brusch RG, Laymon RA, Niesman IR, et al. 2003. Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. Neuron 40:25–40.
90. Piccioni F, Pinton P, Simeoni S, Pozzi P, Fascio U, et al. 2002. Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes. Faseb J 16:1418–1420.
91. Reid E, Kloos M, Ashley-Koch A, Hughes L, Bevan S, et al. 2002. A kinesin heavy chain (*KIF5A*) mutation in hereditary spastic paraplegia (SPG10). Am J Hum Genet 71:1189–1194.
92. Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, et al. 2003. Mutant dynactin in motor neuron disease. Nat Genet 33:455–456.
93. Wilkinson MF, Shyu AB. 2001. Multifunctional regulatory proteins that control gene expression in both the nucleus and the cytoplasm. Bioessays 23:775–787.
94. Izaurralde E, Lewis J, Gamberi C, Jarmolowski A, McGuigan C, Mattaj IW. 1995. A cap-binding protein complex mediating U snRNA export. Nature 376:709–712.
95. Ohno M, Segref A, Bachi A, Wilm M, Mattaj IW. 2000. PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. Cell 101:187–198.
96. Meister G, Buhler D, Pillai R, Lottspeich F, Fischer U. 2001. A multiprotein complex mediates the ATP-dependent assembly of spliceosomal U snRNPs. Nat Cell Biol 3:945–949.
97. Friesen WJ, Dreyfuss G. 2000. Specific sequences of the Sm and Sm-like (Lsm) proteins mediate their interaction with the spinal muscular atrophy disease gene product (SMN). J Biol Chem 275:26370–26375.
98. Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G. 2001. SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell 7:1111–1117.
99. Brahms H, Meheus L, de Brabandere V, Fischer U, Luhrmann R. 2001. Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B′ and the Sm-like protein LSm4, and their interaction with the SMN protein. Rna 7:1531–1542.
100. Friesen WJ, Paushkin S, Wyce A, Massenet S, Pesiridis GS, et al. 2001. The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. Mol Cell Biol 21:8289–8300.
101. Yong J, Pellizzoni L, Dreyfuss G. 2002. Sequence-specific interaction of U1 snRNA with the SMN complex. Embo J 21:1188–1196.
102. Narayanan U, Ospina JK, Frey MR, Hebert MD, Matera AG. 2002. SMN, the spinal muscular atrophy protein, forms a pre-import snRNP complex with snurportin1 and importin beta. Hum Mol Genet 11:1785–1795.
103. Massenet S, Pellizzoni L, Paushkin S, Mattaj IW, Dreyfuss G. 2002. The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. Mol Cell Biol 22:6533–6541.
104. Doussau F, Augustine GJ. 2000. The actin cytoskeleton and neurotransmitter release: an overview. Biochimie 82:353–363.
105. Sankaranarayanan S, Atluri PP, Ryan TA. 2003. Actin has a molecular scaffolding, not propulsive, role in presynaptic function. Nat Neurosci 6:127–135.