Eukaryon

Volume 6 *Evolving Connections* Article 32

3-1-2010

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Summary

Spinal muscular atrophy (SMA) is the most common motor neuron degenerative disease and is the principal genetic cause of infant mortality, affecting 1 in every 6000 newborns. The survival of motor neurons (SMN) gene has been implicated as the disease-causing gene in SMA, and it is deleted or mutated in over 98% of SMA patients. Our lab has pioneered research elucidating the functions of the SMN protein, which we have determined is a part of a large multi-protein complex (the SMN complex) that contains at least seven additional proteins, identified as Gemins 2-7. The SMN complex is essential for the biogenesis of small nuclear RNA ribonucleoproteins (snRNPs), which are the major components of pre-mRNA splicing machinery. We have determined that the genetic aberrations observed in SMA patients result in the depletion of functional SMN within cells. Reduced levels of SMN correspond with a decreased capacity for snRNP assembly and widespread defects in splicing. However, the mechanism by which SMN deficiency and universal defects in splicing lead to the cell-specific phenotype within motor neurons remains unclear. Further research into the role of SMN in spinal muscular atrophy will potentially aid in the development of treatments for this devastating neurodegenerative disease.

Introduction

Spinal muscular atrophy (SMA) is a severe neuromuscular disorder characterized by the degeneration of motor neurons in the spinal cord, resulting in limb and trunk paralysis, muscular weakness, and muscular atrophy (1-3). SMA is the second most common fatal autosomal recessive disorder after cystic fibrosis, affecting approximately 1 in every 6000 newborns (1, 2), and it is classified as the most common genetic cause of childhood mortality (1, 2). Mortality in SMA patients is most often the result of respiratory failure associated with progressive reductions in lung volume caused by paralysis and weakness of intercostal muscles (3). The medical community has recognized three different forms of childhood SMA, which are differentiated based on severity and age of onset: Werdnig-Hoffmann disease (type-I), the intermediate form (type-II), and Kugelberg- Welander disease (type-III), the mildest form (2,3). Currently, treatments for SMA focus on managing the symptoms of the disease in an attempt to prolong life and improve the quality of life of the patient; at the present time, there is no cure for SMA (3).

The survival of motor neurons (SMN) gene has been implicated as the disease-causing gene in all three forms of the disease, and it is deleted or mutated in more than 98% of SMA patients (2). The gene is duplicated as an inverted repeat that is located on human chomosome 5 at 5q13, and SMA results from homozygous deletions or mutations specifically in the telomeric copy of SMN (SMN1) (2, 4). The centromeric copy of the gene, SMN2, produces mostly an alternatively spliced form of the SMN protein, which lacks the amino acids encoded by exon 7 and is unstable and rapidly degraded (2). Hence, SMN2 can only partially compensate for aberrations in the SMN1 gene and therefore does not provide complete protection from SMA. The SMA phenotype is thus believed to be the result of SMN protein deficiency.

The identification of the SMN gene as the disease gene of SMA has led to the intense study of the SMN protein and its functions within the cell. In this review, we will examine important discoveries that have provided valuable insights into the cellular functions of SMN and its role in the pathogenesis of SMA.

SMN and the SMN Complex

The SMN protein is a polypeptide consisting of 294 amino acids that is expressed in all mammalian tissues and in all cell types of vertebrate organisms, but particularly high levels are expressed in motor neurons (5). The presence of SMN is essential for cell viability in all model organisms studied thus far, including mice, C. elegans, and S. pombe (6-8). SMN is found in both the cytoplasm and the nucleus of somatic cells and is highly concentrated in nuclear structures called gems (9). Gems are similar in size and number to Cajal (coiled) bodies and are often located near or associated with these structures in the nucleus (9); this suggests that gems and Cajal bodies may have related functions. Cajal bodies are believed to be important in the metabolism of small nuclear ribonucleoproteins (snRNPs) and are therefore thought to contribute to pre-mRNA processing (10). The discovery that SMN is associated with gems and Cajal bodies was one of the first clues that the SMN protein may be involved in snRNP processing and that SMA may arise from defects in RNA metabolism (9).

Further research into the SMN protein revealed that it is part of a large multi-protein complex (the SMN complex) that contains at least seven additional proteins, identified as Gemins2-8 (Figure 1) (11-16). All of the Gemins colocalize with SMN in the cytoplasm, the nucleoplasm, and in gems (17). SMN is always tightly associated with Gemin2, which was originally identified as SIP1 (SMN interacting protein 1) (11, 18). Interestingly, Gemin2 has significant sequence similarity with Brr1, a protein found in yeast that is necessary for the formation of snRNPs (18). SMN also associates with Gemin3, a DEAD-box RNA helicase that mediates the interaction of SMN with Gemin4 (12, 19). Surprisingly, in addition to colocalizing with SMN in the cytoplasm and in gems, Gemin4 is also detected in the nucleolus (12). Another integral component of the SMN complex is Gemin5, which is a large tryptophan-aspartic acid (WD) repeat protein that binds SMN directly (13). Gemins6 and 7 also associate with SMN as a part of the SMN complex, with Gemin7 facilitating the interaction of Gemin6 with the SMN complex (14-15). A protein of unknown function, Gemin8, interacts directly with the heterodimer consisting of Gemins6 and 7 and is believed to be the final protein constituent of the SMN complex (16).

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*This author wrote the paper for Biology 480: Neural Frontiers (Senior Seminar) taught by Dr. Shubhik DebBurman.

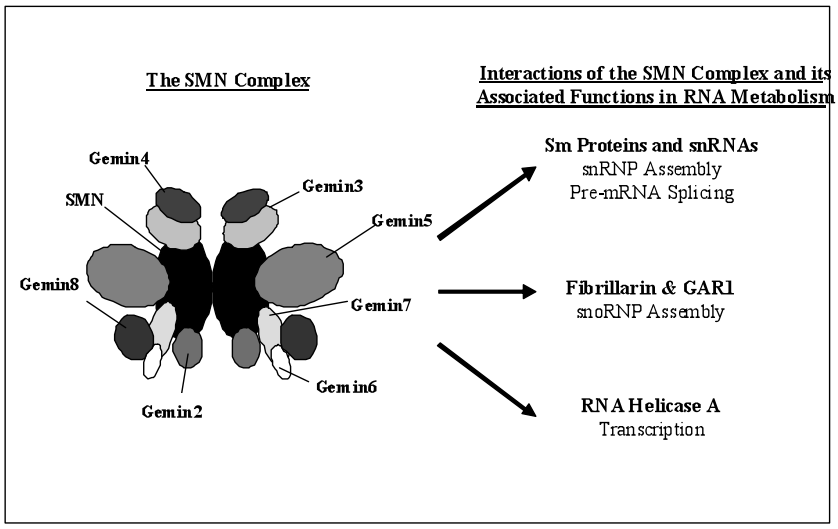

Figure 1. The SMN Complex and Its Functions As depicted on the left, the SMN complex is a large, oligomeric multi-protein complex. The complex consists of SMN as well as at least seven other proteins shown as Geminsβ. For simplicity, the SMN complex is shown here as a dimmer. There are several proteins that directly interact with the SMN complex, and these interactions are the basis of the complex's numerous intracellular functions associated with RNA metabolism. Specifically, the SMN complex has an essential function in snRNP assembly and premRNA splicing, which are mediated by its interactions with Sm proteins and snRNAs. Additionally, the complex binds to the proteins fibrillarin and GAR1 and thus has a role in snoRNP biogenesis. Finally, the complex interacts with RNA helicase A and therefore contributes to RNA polymerase II transcription. Adapted from Battle et al., 2006.

Functions of the SMN Complex

In order to elucidate the molecular mechanisms that lead to the development of SMA, it was first necessary to identify the cellular functions of the SMN protein and the SMN complex. Through extensive research, we have determined that SMN is involved in a multitude of intracellular processes, and that the SMN complex has a fundamental role in cellular RNA metabolism (Figure 1).

snRNP Assembly and Pre-mRNA Splicing

Our investigations of the cellular functions of SMN demonstrated that it is essential for the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs) (11, 14, 17-18, 20-23, 26). snRNPs are the major components of the spliceosome, the machinery responsible for carrying out pre-mRNA splicing (21). The biogenesis of snRNPs is a complex process that requires multiple steps in both the cytoplasm and the nucleus (22). Each spliceosomal snRNP consists of one or two small nuclear RNAs (snRNAs), several proteins specific to individual snRNAs, and a set of seven common proteins called Sm proteins (21). The major snRNAs utilized in snRNP formation are identified as U1, U2, U5, and U4/6 (21). In the cytoplasm, the Sm proteins form a seven-membered ring around a specific sequence on each snRNA called the Sm site, thus forming the Sm core (21-23). This is followed by hypermethylation of the 5' cap to form the 2,2,7-trimethyl guanosine cap. (22). The Sm core and the methylated cap help recruit the molecules required for transporting the snRNPs into the nucleus, where they function in splicing (21).

Our research has led to numerous important findings that have provided evidence that the SMN complex is directly involved in the biogenesis of spliceosomal snRNPs. First, we found that the SMN complex interacts directly with both spliceosomal snRNP (Sm) proteins and spliceosomal snRNAs (11, 18). SMN interacts with the arginine- and glycine-rich (RG) domains of Sm proteins via its carboxyl-terminal YG domain (18). The association of the SMN complex with the Sm proteins is enhanced by the methylation of specific arginines within the RG domains by the mehtylosome/PRMT5 complex (24). Each of the Gemins, except Gemin2, directly binds to Sm proteins, as well (11-16). In the cytoplasm, the SMN complex assembles Sm proteins onto the Sm site of snRNAs in an ATP-dependent reaction, thus forming the Sm core (14, 24). Importantly, we found that the extent of Sm core assembly on snRNAs is directly proportional to the amount of SMN protein in the cell (25).

Independent of its interactions with Sm proteins, the SMN complex also binds directly to snRNAs (14, 17, 26-28). We have determined that Gemin5 is the specific component of the complex that binds and identifies snRNAs and allows the SMN complex to assemble them into snRNPs (29). Additionally, we have identified specific sequences and structural features that are required for binding of the SMN complex to snRNAs (27-28). The minimum requirements for binding of the SMN complex to the snRNAs are the Sm site as well as a SMN complex binding region (28). For all but one of the snRNAs, the SMN complex binding domain consists of at least one stem-loop immediately 3' of the Sm site (28). In addition, the SMN complex specifically recognizes the first adenosine and the first and third uridines of the Sm site in all snRNAs (26). These characteristics constitute a code that is recognized by the SMN complex and specifies which cellular RNAs are snRNAs that should be utilized for snRNP assembly (26).

The SMN complex performs an essential function within the cell by ensuring that Sm cores assemble only on the correct RNA targets (14). The SMN complex accomplishes this by identifying the snRNAs required for snRNP assembly based on the specific sequences and structural features described above (26). Moreover, the SMN complex binds the Sm proteins so that there are no free proteins that can assemble outside of the SMN complex (14). The bound Sm proteins are therefore only capable of assembling an Sm core on RNAs that also bind to the SMN complex (14). The high specificity of snRNA binding to the SMN complex ultimately results in the delivery of Sm proteins only on the appropriate snRNAs (14). In preventing the nonspecific assembly of Sm cores on random RNAs, the SMN complex serves as a specificity factor that ensures that

only the correct snRNPs are formed, which is a vital function within cells.

Through extensive research, we have discovered additional evidence supporting the role of the SMN complex in snRNP biogenesis. For example, inhibition of the SMN complex interferes with the formation of snRNPs from Sm proteins and spliceosomal snRNAs and disrupts the import of the mature snRNP into the nucleus (11, 30). Moreover, the SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway, which suggests that it may have multiple functions throughout this process (31). The complex binds newly exported snRNAs and remains associated with the snRNPs during Sm core assembly, hypermethylation of the 5' cap, and binding to the machinery required for reimport of the snRNP into the nucleus (31). These findings provide further support for the hypothesis that the SMN complex is essential for snRNP biogenesis in the cytoplasm.

Furthermore, SMN is necessary for snRNP function and is required for pre-mRNA splicing in the nucleus (30). Wild-type SMN simulates splicing in vitro, whereas dominant-negative SMN mutants inhibit pre-mRNA splicing (30). Similarly, the addition of anti-SMN antibodies to nuclear extracts inhibits splicing (30); a role for SMN in splicing is also suggested by its interaction with a family of hnRNP proteins, referred to as hnRNP Q proteins, which are components of the spliceosome and are required for efficient pre-mRNA splicing in vitro (32). Although these findings indicate a functional role for the SMN complex in pre-mRNA splicing, it is unclear whether it is directly or indirectly involved in the process. It is possible that the function of SMN in splicing may be secondary to its function in snRNP biogenesis (32). However, we hypothesize that SMN facilitates pre-mRNA splicing by regenerating or recycling snRNPs in the nucleus (30). Based on these data, we conclude that the SMN complex has a fundamental role in snRNP assembly and pre-mRNA splicing and is therefore vital for the production of mature mRNA and overall cellular function.

Additional Functions of the SMN Complex

Through the course of our studies, we have identified several additional functions for the SMN complex in RNA metabolism. For example, SMN binds directly to fibrillarin and GAR1, which are proteins associated with the two different classes of small nucleolar ribonucleoproteins (snoRNPs) that are involved in the posttranscriptional processing of ribosomal RNA (33). Overexpression of a dominant-negative mutant of SMN causes massive reorganization of snoRNPs and inhibits transcription within the nucleolus (33). These findings indicate that the SMN complex is involved in snoRNP assembly and metabolism and may suggest that the SMN complex is necessary for the biogenesis and function of a diverse array of ribonucleoprotein complexes.

The SMN complex also associates with RNA helicase A (RHA), a DEAH box RNA helicase that binds RNA polymerase II (34). Expression of the dominant-negative SMNΔN27 produces a drastic rearrangement of RNA polymerase II and RHA within the nucleus and results in the inhibition of transcription by RNA polymerases I and II (34). These findings suggest that the SMN complex may have a role in transcription, particularly in the assembly of the RNA polymerase II transcription and processing machinery (34).

The Role of SMN in SMA

Spinal muscular atrophy is caused by genetic deletions and mutations in the SMN1 gene that result in the depletion of functional SMN protein within cells (Figure 2) (2, 5). Studies of cells derived from SMA patients have shown that the severity of SMA phenotypes is inversely correlated with the amount of SMN protein in these cells (5). Individuals affected by the most severe form of SMA (type-I) have barely detectable levels of the SMN protein in various cell types, including motor neurons (5, 35). In SMA, reduced levels of SMN correspond with defects in RNA metabolism and RNP biogenesis (19-20, 25, 30, 36).

Defects in snRNP Assembly and Splicing

SMA patients with deletions and mutations in the SMN1 gene produce SMN proteins that are not functional, resulting in defects in snRNP assembly and pre-mRNA splicing (19-20, 25, 30, 36). Through the course of our research, we have determined that mutations in the SMN protein interfere with its ability to oligomerize, as demonstrated by a decreased capacity for SMN from SMA patients to interact with Gemin3 (19, 35). This indicates that SMN mutants may have a reduced ability to form the complete SMN complex, which may contribute to the functional deficits observed in SMA patients.

We have also found that mutant SMN proteins derived from SMA patients are defective in binding to Sm proteins and associating with snRNPs (20). Additionally, we have established that the reduction of SMN protein levels in cells diminishes their ability to produce snRNPs and that the rate of snRNP biogenesis is significantly reduced in cells with low levels of SMN (25). This is consistent with the finding that cellular extracts from SMA patients have a lower capacity for snRNP assembly that is directly proportional to the reduction of SMN protein in these cells (25). Due to its role as a specificity factor for the assembly of snRNPs, it is also possible that SMN protein deficiency also results in the nonspecific binding of Sm proteins to RNAs, which would ultimately result in the formation of aberrant RNPs (14). These findings are particularly significant because they suggest that snRNP assembly may provide a molecular biomarker for SMA, which could aid in clinical trials, the development of drug therapies, and investigations into the disease pathology (37).

Our studies have also revealed a link between SMN deficiency in SMA and defects in pre-mRNA splicing. Importantly, SMN mutants found in SMA patients cannot stimulate splicing and therefore lack the ability to regenerate splicing machinery (30). Furthermore, we recently discovered that SMN deficiency in mice comparable to that which occurs in severe SMA causes cell type-specific disruptions in the repertoire of snRNAs and results in widespread defects in pre-mRNA splicing (36). Interestingly, the splicing defects were observed in numerous different mRNAs from multiple tissues of the SMA mice models (36). These surprising discoveries support the hypothesis that the SMN complex is a major factor in RNA metabolism and splicing regulation and provide evidence that SMA is a general splicing disease that does not exclusively affect splicing in motor neurons (36).

SMN Deficiency and Effects in Motor Neurons

The mechanism by which SMN deficiency leads to the cell-specific phenotype within motor neurons has not been established, although some lines of evidence provide insights into this phenomenon. In humans, the small amount of full length SMN produced by SMN2 is sufficient for viability of all cells except motor neurons, which suggests that motor neurons are particularly sensitive to SMN reduction (5). This observation gives rise to the possibility that SMN may have unique functions in motor neurons, which has been supported by numerous findings in other laboratories (38-40). One recent discovery was that the axonal-SMN protein,

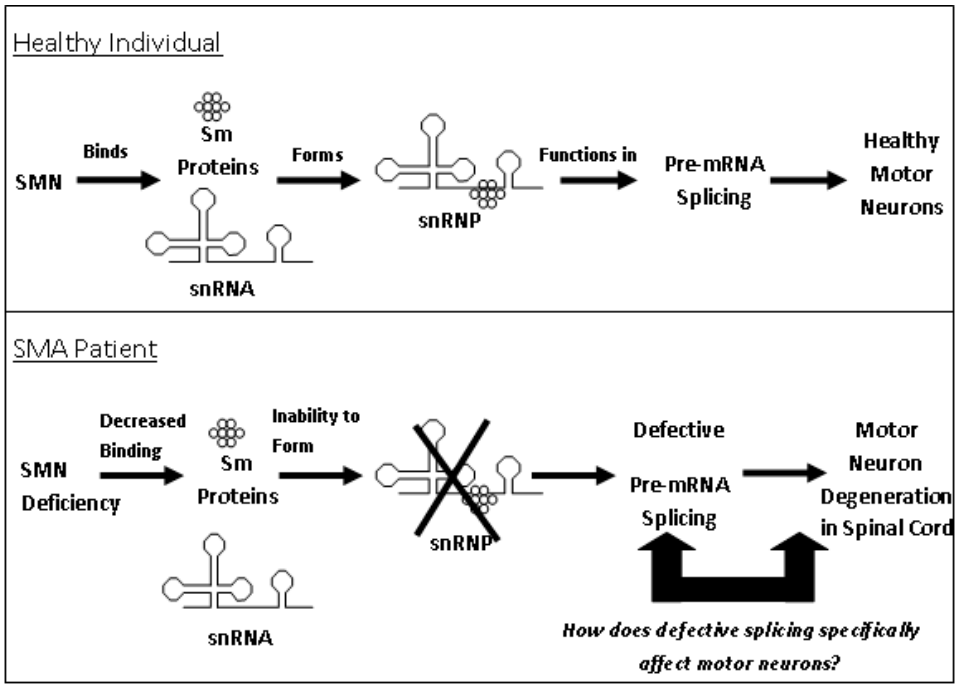

Figure 2. The Role of SMN Deficiency in SMA: A model of the normal function of SMN in snRNP formation and pre-mRNA splicing in healthy individuals (top) and the functional defects observed in spinal muscular atrophy (bottom). In SMA, SMN deficiency leads to decreased binding of SMN to Sm proteins and snRNA, which results in an inability to form snRNPs and defective splicing of pre-mRNA. This produces the SMA phenotype, which is characterized by degeneration of motor neurons in the spinal cord. The mechanism by which universal defects in splicing lead to attrition of motor neurons specifically is still not understood, although it is hypothesized that cell type-specific differences in splicing defects may provide a possible explanation.

a protein isoform produced from the SMN gene, is selectively expressed in developing motor neurons, is involved in axonogenessis, and is mainly localized in axons (40). These findings could potentially explain the physiological effects of SMN deficiency in SMA patients since the depletion of the axonal-SMN protein isoform in motor neurons may have dramatic effects on their survival (40). Additional research has demonstrated that SMN also plays a role in axonal growth, neuromuscular junction formation, and the transport of RNA along axons (41-42). Defects in any of these processes as a result of SMN deficiency in SMA could result in the selective death of motor neurons.

Based on our current understanding of SMN function, however, it is reasonable to conclude that defects in RNA metabolism contribute to disease pathogenesis. Furthermore, the discovery that SMN deficiency causes tissue-specific perturbations in snRNAs and splicing suggests a possible explanation for the selective degeneration of motor neurons, which may experience more defects in splicing than other cell types (36). Low levels of SMN in SMA may therefore result in specific defects in RNA processing within motor neurons that disrupt their development and survival (36). The identification of specific aberrations in RNA metabolism and pre-mRNA splicing within motor neurons will provide further support for this hypothesis and thus will likely be one of the focal points of future research in the field.

Discussion

Since the discovery in 1995 that mutations and deletions in the survival of motor neurons (SMN) gene cause spinal muscular atrophy (2), there have been profound advances in our understanding of the SMN protein and its role in SMA; namely, we have determined that SMN exists as a part of a multi-protein complex (the SMN complex), which has an essential role in pre-mRNA splicing and the assembly of snRNPs (11, 14, 17, 30). Furthermore, we have established that SMA pathogenesis is caused by drastic reductions in SMN protein levels, resulting in defects in SMN complex function (19-20, 25, 30). Importantly, we have also found that SMN deficiency causes cell type-specific changes in snRNP assembly and splicing, which may help explain the restricted death of motor neurons in SMA (36). Hence, our studies have provided valuable insights into the molecular functions of the SMN protein in cells, the cellular pathways that are affected by decreased expression of functional SMN, and the mechanisms that may be contributing to the selective degeneration of motor neurons.

Moreover, through the recognition that SMA is caused by SMN protein deficiency, our work has provided innovative treatment options for SMA, such as the development of agents that increase the availability of SMN (3). Pharmaceuticals that could potentially enhance SMN2 transcription, for example, could help compensate for mutations in the SMN1 gene by providing more functional SMN protein (3). Additional approaches to developing prospective therapeutic intervention in spinal muscular atrophy include methods used in other neurodegenerative diseases, such as gene therapy and cell replacement therapy (3). Further research and the identification of the specific molecular pathways contributing to SMA should continue to pave the way for the development of new therapeutic approaches for treating this devastating neurodegenerative disease.

Despite these major advancements, however, numerous questions about SMN and its involvement in SMA pathogenesis remain unanswered, such as the specific factors that result in the motor-neuron selective phenotype and the molecular basis for widespread splicing defects caused by SMN deficiency. Ideally, elucidating the

remaining areas of inquiry through continued research will provide additional therapeutic targets for SMA as well as fundamental knowledge regarding motor neuron biology and pathology.

Acknowledgements

I would like to thank Lake Forest College for providing the resources and facilities required to aid me in this endeavor. I would also like to thank Dr. DebBurman for his constant guidance and support, as well as Danielle Clark for her assistance and advice in writing this review.

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