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Molecular Functions of the SMN Complex

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The SMN complex is essential for the biogenesis of spliceosomal small nuclear ribonucleoproteins and likely functions in the assembly, metabolism, and transport of a diverse number of other ribonucleoproteins. Specifically, the SMN complex assembles 7 Sm proteins into a core structure around a highly conserved sequence of ribonucleic acid (RNA) found in small nuclear RNAs. The complex recognizes specific sequences and structural features of small nuclear RNAs and Sm proteins and assembles small nuclear ribonucleoproteins in a stepwise fashion. In addition to the SMN protein, the SMN complex contains 7 additional proteins known as Gemin2-8, each likely to play a role in ribonucleoprotein biogenesis. This review focuses on the current understanding of the mechanism of the role of the SMN complex in small nuclear ribonucleoprotein assembly and considers the relationship of this function to spinal muscular atrophy.

Keywords: spinal muscular atrophy; ribonucleoproteins; Gemin2-8

Mutations in the survival motor neuron gene (*SMN*) that decrease expression of the functional SMN protein result in spinal muscular atrophy. Within the pediatric population, spinal muscular atrophy can be included in the list of single gene defect diseases where the loss of a single essential protein expressed in all tissues and cells results in the selective death of a single cell type. In the case of spinal muscular atrophy, this is the lower motor neuron. As has been noted elsewhere, another example of this type of disease is Rett syndrome, which results from mutations in a transcriptional repressor that is also ubiquitously expressed.[1] Like Rett syndrome, the basis of cell selectivity in spinal muscular atrophy is not clear. Spinal muscular atrophy also shares similarities (and, consequently, pathogenic riddles) with several adult neurodegenerative disorders that affect motor neurons, including amyotrophic lateral sclerosis, primary lateral sclerosis, hereditary spastic parapareses, and the distal hereditary neuropathies. Thus, lessons from the study of the molecular pathogenesis of spinal muscular atrophy may provide important insights into other neurogenetic diseases.

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Since 1995, when mutations in *SMN* were shown to cause spinal muscular atrophy,[2] there has been great progress toward understanding the molecular functions of the SMN protein in cells, the cellular pathways that are affected by decreased expression of SMN, and the design of potential therapeutic strategies to treat patients with this disease. Several recent reviews have discussed SMN function.[1,3-6] Here we focus on the function of SMN in spliceosomal ribonucleoprotein biogenesis and on how defects in this function may result in spinal muscular atrophy.

The SMN Complex

The SMN protein is a 294–amino acid polypeptide that is expressed in all metazoans and in all cell types of vertebrate organisms. Biochemically, SMN does not appear to exist within cells in isolation but instead forms part of a large protein complex, the SMN complex. The SMN complex is composed of the SMN protein and 7 additional proteins, Gemin2-8.[7-14] The Gemins bind to and colocalize with SMN in the cytoplasm and in discrete nuclear bodies called gems.[11] The SMN protein is an essential protein in all cell types studied thus far. Complete knockout of SMN results in embryonic lethality in divergent organisms, including human, mouse, chicken, and *Caenorhabditis elegans* and is also lethal in *Schizosaccharomyces pombe*. In experimental systems, SMN expression appears necessary for cell survival regardless of cell type; therefore, a major mystery of the pathogenesis in spinal muscular atrophy is the basis of motor neuron–specific cell loss with relative depletion of SMN throughout the body.

The SMN complex is large (40S to 80S in sucrose gradient sedimentation) and is salt resistant (750-mmol/L NaCl)[4]; however, under less stringent conditions, the SMN complex is bound to several proteins, including the spliceosomal Sm proteins.[15,16] Insights into the possible cellular functions of the SMN complex have been derived from a detailed analysis of the substrate proteins and small nuclear ribonucleic acids (RNAs) that interact with the SMN complex.[17,18] The function of the SMN complex in spliceosomal small nuclear ribonucleoprotein assembly is discussed in detail later here and serves as an example of SMN function; however, the SMN complex likely also functions in the assembly, metabolism, and transport of diverse classes of ribonucleoproteins, including small nucleolar ribonucleoproteins, telomerase ribonucleoprotein, microribonucleoproteins, and the machineries that carry out transcription and premessenger RNA splicing.[18-29]

Spliceosomal U Small Nuclear Ribonucleoprotein Assembly

The premessenger RNA splicing process is essential for the successful execution of eukaryotic gene expression and is carried out by the spliceosome in the nucleus. The major components of the spliceosome are the U1, U2, U5, and U4/U6 small nuclear ribonucleoproteins.[30] Each of the small nuclear ribonucleoproteins (except for U6) is composed of 1 small nuclear RNA molecule, a set of 7 common proteins, and several proteins specific to individual small nuclear RNAs.[30-32] A crucial aspect of small nuclear ribonucleoprotein biogenesis is that although assembly of these components can occur spontaneously in vitro, within cells, the process is highly regulated and requires both adenosine triphosphate hydrolysis and dedicated assembly factors.[15,33] Small nuclear ribonucleoprotein biogenesis begins with the transcription of the U small nuclear RNAs in the nucleus, followed by their export to the cytoplasm, where the major assembly of the small nuclear ribonucleoproteins occurs. The common proteins, called Sm proteins (B/B', D1, D2, D3, E, F, and G), are arranged into a stable heptameric ring, the Sm core, on a highly conserved, uridine-rich sequence motif, the Sm site, of the small nuclear RNAs.[34-36] After assembly, the 7-methyl guanosine cap of the small nuclear RNA is modified into a 2,2,7-trimethyl guanosine cap, and the properly assembled and modified small nuclear ribonucleoproteins are then imported into the nucleus, where additional small nuclear ribonucleoprotein-specific proteins associate to form fully functional small nuclear ribonucleoproteins.[30,37-41] Mature small nuclear ribonucleoproteins then carry out the process of premessenger RNA splicing. Clearly, to ensure that splicing is carried out in an efficient and timely manner, the process of small nuclear ribonucleoprotein assembly must run smoothly and efficiently.

How do cells ensure that Sm proteins will be assembled only onto the correct small nuclear ribonucleoprotein? This is where the SMN complex is essential. The SMN complex directly recognizes and binds to both the protein and the RNA components of the ribonucleoproteins and facilitates their interaction, thereby ensuring a strict specificity of the small nuclear ribonucleoprotein assembly process (Figure 1).

To enforce specificity, the SMN complex must be able to specifically bind to Sm proteins. In fact, each component of the SMN complex, except Gemin2, binds directly to Sm proteins. Gemin6 and Gemin7 were both recently shown to have structures similar to Sm proteins, and reduction of Gemin6 by RNA interference disrupted the ability of the SMN complex to bind Sm proteins. In addition, the SMN protein itself binds to arginine- and glycine-rich domains found in the Sm proteins B, D1, and D3. This interaction is enhanced by a posttranslational, symmetric dimethyl arginine modification that occurs at specific arginines in the glycine-rich domains of the Sm proteins—a reaction carried out by the 20S methylosome that contains an arginine methyltransferase called JBP1 or PRMT5.[17] Clearly, the SMN complex is a machine designed to bind Sm proteins.

The SMN complex also binds directly and with sequence specificity to the spliceosomal U small nuclear RNAs.[16,42] The minimal SMN complex–binding domain in small nuclear RNAs, except U1, is composed of an Sm site (AUUUUUG) and an adjacent 3' stem loop (Figure 2). A detailed analysis of this binding demonstrated that although the Sm site is recognized by virtue of its sequence, the specific sequence of the adjacent 3' stem loop is not important, provided that it is located within a short distance of the 3' end of the RNA and that the stem contains from 7 to 12 base pairs and the loop contains 4 to 17 nucleotides.[42] These sequence and structural features appear to be uniquely present within U small nuclear RNAs (interestingly, they are also present in the small nuclear RNA encoded by the lymphotrophic *Herpesvirus saimiri*[43]); therefore, the SMN complex scrutinizes RNAs that contain an Sm site, identifies the spliceosomal small nuclear RNAs by recognizing the small nuclear ribonucleoprotein code contained within them, and then assembles Sm proteins onto them.

It is now apparent that the Gemins, not only the SMN protein, are essential to the process of small nuclear ribonucleoprotein assembly. Recently, Gemin5 was identified as the small nuclear RNA-binding protein of the SMN complex.[44] Gemin5 directly and specifically binds to the small nuclear ribonucleoprotein code at the 3' end of small nuclear RNAs. By providing the SMN complex with the ability to bind to this class of RNAs, Gemin5 serves to specify small nuclear RNAs, among all cellular RNAs, for assembly into functional small nuclear ribonucleoproteins. Gemin5 is a 175-kDa protein with no recognizable RNA-binding motif. Rather, the N-terminal half

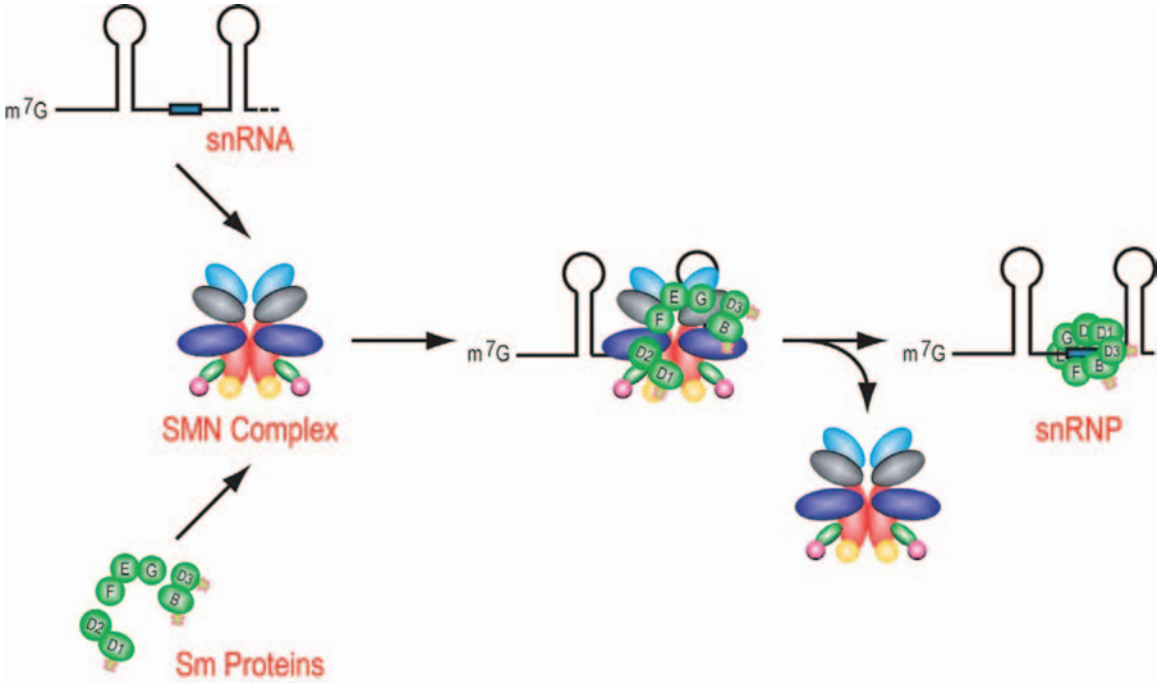

Figure 1. Schematic of the function of the SMN complex in spliceosomal small nuclear ribonucleoprotein assembly. The SMN complex directly binds both Sm proteins as well as small nuclear ribonucleic acids and assembles the Sm proteins onto the ribonucleic acid Sm site (discussed in the text).

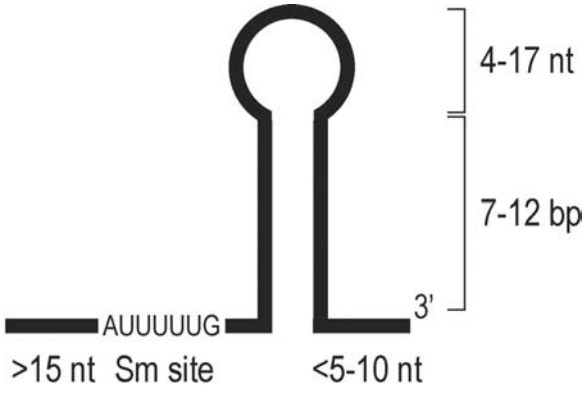

Figure 2. The small nuclear ribonucleoprotein code. The defining characteristics of small nuclear ribonucleic acids that are recognized by direct binding of the SMN complex. The minimal domain is composed of an Sm site and an adjacent 3′ stem loop. For the SMN complex to bind, the adjacent 3′ stem loop must be located within a short distance of the 3′ end of the ribonucleic acid; however, the exact sequence of this structure can vary.

of Gemin5 contains 13 WD repeats, whereas the C-terminal half shows no homology with any other known proteins. As such, Gemin5 seems to be unique among RNA-binding proteins.

The capacity of a cell to assemble small nuclear ribonucleoproteins can now be measured quantitatively, and it is proportional to the expression levels of the SMN protein in the cell.[45] In cells expressing low levels of SMN, either through mutation of *SMN* (ie, spinal muscular atrophy) or through RNA interference in experimental systems, small nuclear ribonucleoprotein assembly is correspondingly decreased. The ability to measure small nuclear ribonucleoprotein assembly capacity is not strictly a measure of the expression of SMN because decreased assembly is also seen in cells depleted of other Gemins. In experimental systems, a reduction of all other Gemins also decreases small nuclear ribonucleoprotein assembly.[14,44,46] An important observation is that small nuclear ribonucleoprotein assembly is also decreased in cell lines derived from spinal muscular atrophy patients[45]; therefore, small nuclear ribonucleoprotein assembly measurements in patient cells provide an additional molecular biomarker for use in clinical trials, drug discovery experiments, and pathophysiologic investigations.

Spinal Muscular Atrophy–Relevant SMN Function

How does a loss of a general function, small nuclear ribonucleoprotein assembly capacity result in the selective loss of

motor neurons in spinal muscular atrophy? Much work is currently under way to address this question. In mice, small nuclear ribonucleoprotein assembly varies markedly in different tissues and during development.[47] In the mouse spinal cord, small nuclear ribonucleoprotein assembly activity is highest during embryonic development and then declines after the first week of life.[47] This temporal pattern coincides with the onset of myelination in the central nervous system and suggests that high levels of small nuclear ribonucleoprotein biogenesis are required for neuronal development. In the embryonic zebrafish, motor neuron development is altered by reduction of SMN and can be rescued by injection of formed small nuclear ribonucleoproteins[48]; therefore, it is possible that reduced SMN protein leads to reduced small nuclear ribonucleoprotein production, which leads to changes in the ability of cells to splice genes correctly.[3,5] Because each cell is faced with the execution of a unique constellation of splicing events on a unique set of premessenger RNAs, it appears likely that the splicing of specific premessenger RNAs that are crucial for motor neuron survival and/or development may be altered in spinal muscular atrophy; however, specific alterations in messenger RNAs from spinal muscular atrophy patients or model systems have so far not been identified.

It has already been stressed that SMN function in cells is not likely to be limited to its role in spliceosomal ribonucleoprotein assembly. SMN accumulates in the axons of motor neurons (and in other types of neurons) during nervous system development and thus may also have functions that are unique to neurons.[47,49-51] For example, it has been suggested that SMN may function as a molecular chaperone for β-actin messenger RNA localization in motor axons.[52] Given the large number of RNA and protein binding partners of SMN and the SMN complex, it is likely that the number of cellular functions attributed to the SMN complex will continue to increase; however, based on our current understanding, it is reasonable to conclude that the pathologic consequence of low SMN levels in spinal muscular atrophy is the disruption of normal cellular RNA metabolism required for motor neuron development and survival. The identification of specific defects in RNA metabolism, such as the identification of specific splicing defects in genes that result in motor neuron loss in spinal muscular atrophy, may provide additional therapeutic targets for this disease and will provide fundamental insights into motor neuron biology and pathology.

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