Correct Splicing: The Desolation of SMA

Interactions Between Gemin-2 and SMN

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Abstract

Spinal muscular atrophy (SMA) is a genetic disorder often leading to death before age two. These deaths are caused by decay of motor neurons in the spine, affecting one in six thousand babies yearly (Families of SMA, 2013). It is unknown why a mutation or deletion of the SMN1 gene, which produces survival motor neuron (SMN) protein, causes this. The SMN complex is made of SMN and smaller units called Gemin proteins. In a normal system, the SMN1 gene codes for SMN proteins that are part of the SMN complex that form small nuclear ribonucleoproteins (snRNPs) from Sm proteins and sRNA. The SMN protein binds to Gemin-2 which holds five of the seven Sm proteins, the smaller units in snRNPs, in place until the target snRNA sequence is located. The final Sm proteins are added when the N-terminus of Gemin-2 moves. The snRNPs have many functions in cells, five of them being involved in RNA splicing. The knowledge available on normal interactions of SMN and Gemin-2 allow modeling of these proteins through 3D printing by the Hartford Union SMART (Students Modeling A Research Topic) Team. In cases of SMA, the SMN protein cannot bind to Gemin-2 because Asp44 is replaced by valine, causing a break in the ionic bond holding the helices together. While this situation still produces normally operating snRNPs, there are too few to correctly splice the pre-mRNA, leading to SMA.

What is SMA?

Spinal muscular atrophy (SMA) is a genetic disorder caused by a mutation on the SMN1 gene which produces survival motor neuron protein (SMN). SMN proteins are found all over the body and are essential to proper splicing of RNA and the well-being of motor neurons. However, there are not enough SMN proteins being produced in cases of SMA causing problems in splicing. This can leave cells unable to cut introns out of a genetic sequence and paste the exons back together. It is an autosomal recessive disorder, meaning those with the disorder have two recessive alleles. About one in forty people are carriers of the disorder (Families of SMA, 2014). There are four grades of the disease, and children who have grade one will often die before reaching age two. SMA is characterized by the degeneration of motor neurons in the spine, but research has been unable to find the exact reason the spinal cord is targeted. The disorder can affect each person differently, but usually presents with muscle weakness in the legs. Although this weakness may spread over time, SMA is not classified as a progressive disorder. There may also be atrophy of the muscles involved in breathing and eating, leading to more life threatening complications like lung infections and respiratory problems during sleep. While there is no cure for SMA, the skeletal and muscular problems that accompany it can be corrected using braces and other supports, as well as corrective surgery. Children diagnosed with the most severe form of SMA, type one, will usually die before age two. However, the prognosis for those with type four is much more optimistic and they do not normally see any reduction in life span because it presents later In life.

The SMN complex usually splices mRNA by the following process (see "An Inside Look at SMA"):
A. Sm proteins and the SMN complex are located in the cytoplasm. Some SMN complexes can be found in the nucleus as well. The SMN complex preforms splicing functions in the cell.
B. Sm proteins bind to SMN complex and form a pentameter.
C. snRNA is made in the nucleus and transported into the cytoplasm.
D. snRNA binds to SMN complex.
E. Sm proteins form a pentameter around snRNA andGemin-2 holds its N-terminus in place, blocking the formation of the ring around the snRNA. The correct sequence is located and the ring is completed with the final two Sm proteins after the N-terminus moves. This forms a snRNP.
F. The SMN complex and snRNP are transported into the nucleus
G. The introns are spliced out of pre-mRNA. This forms mRNA.
H. The mRNA leaves the cell and can then be used in transcription. During this process the ribosome construct a chain of amino acids which form a polypeptide chain and then form a protein.

When this process is not completed correctly, errors in mRNA splicing occur. Proteins that are produced from incorrectly spliced mRNA may not fit the function they were originally intended to do.

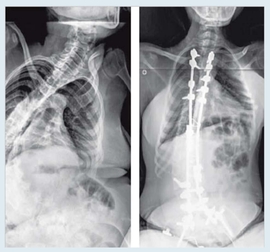
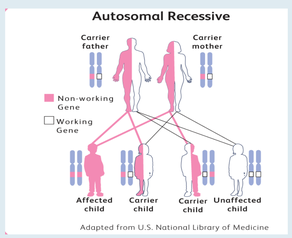


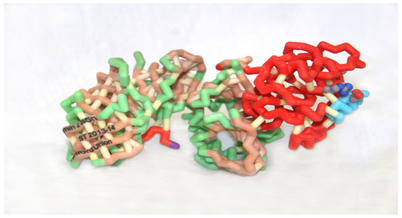
Figure One
This photograph shows a case of type two spinal muscular atrophy where the spine was bent 111 degrees. To correct this, steel was attached to the vertebrae and the pelvis. Children are usually diagnosed using EMGs, genetic testing, and abnormal physical appearance accompanied with delay in development.

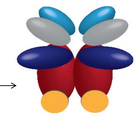
Figure Two
SMA is an autosomal recessive genetic disorder. If both parents are carriers, there is a twenty five percent chance that their child will have the disorder. Children will only have the disease if two recessive genes are inherited. There is a twenty five percent chance that the child will not be a carrier and a fifty percent chance that the child will be a carrier of the disorder.



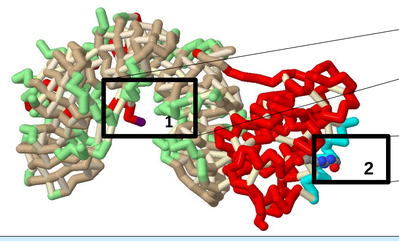
Structure of Gemin-2 and SMN

3S6N.PDB
The model includes the SMN protein (aqua), Gemin-2 (red), and the pentameter of Sm proteins (green, tan). This is only a small portion of the entire SMN complex.





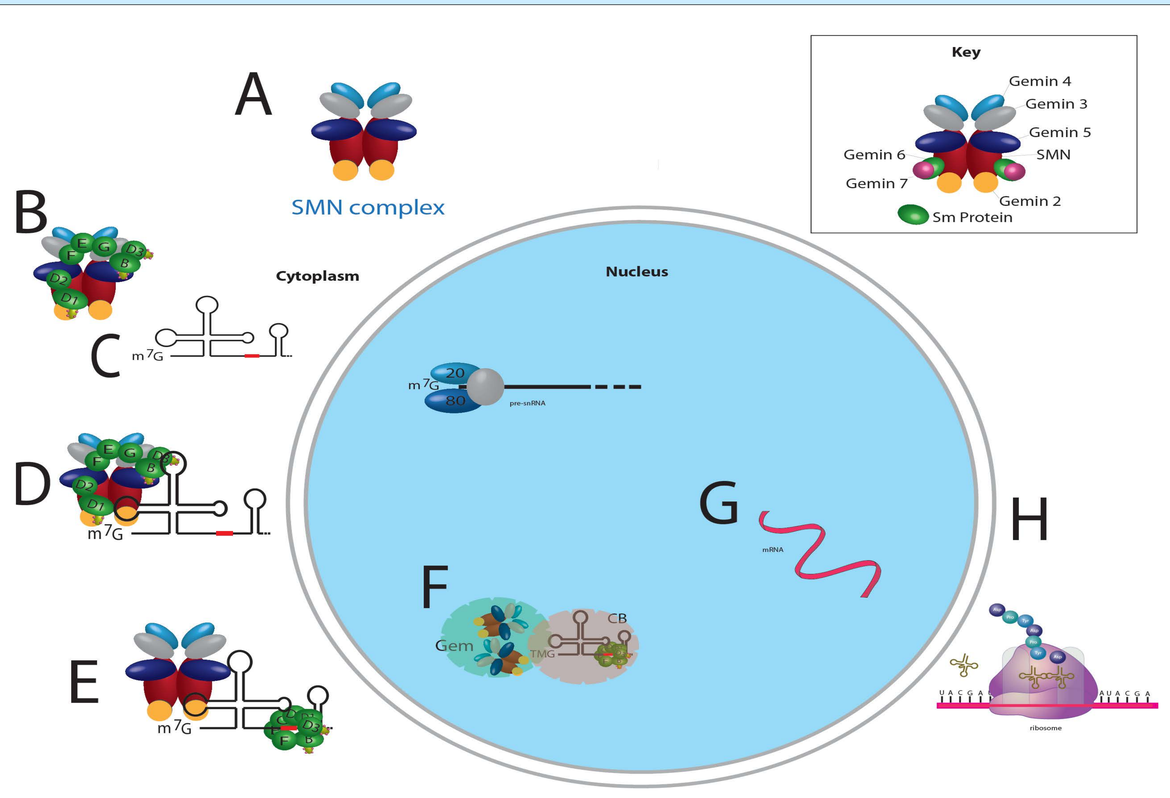
The SMN complex is the vital machinery that builds snRNPs which splice the pre-mRNA that is needed to build proteins. When the complexes were first discovered, they were always very close to known structures in the nucleus so they were called "gems" after the term gemini. The SMN complex is a very large structure comprised of several smaller proteins including Gemin 1, 2, 3, 4, 5, 6, 7, and the SMN protein. It forms a ring using the Sm proteins D1, D2, F, E, G, D3, and B.



1. Gemin-2 is known to hold its N-terminus around the pentameter, as pictured, to block binding to snRNA sequences. The pentameter is comprised of five Sm proteins, forming the incomplete ring that is blocked by Gemin-2. The arm of Gemin-2 moves once the correct sequence is found and the pentameter can be completed with the final two Sm proteins. The completed ring is formed around snRNA to form the snRNPs (small nuclear ribonucleoproteins).

2. The SMN protein binds to Gemin-2 by an ionic bond composed of Arg213 and Asp44, holding the complex together. In cases of SMA, the Asp44 is replaced with a valine and the ionic bond is disrupted. This results a decreased production of functioning snRNPs and the degeneration of motor neurons in the spine.

An Inside Look at SMA



A Pathological Mutation in SMN

This experiment focused on the interactions between the Gemin2 and SMN complex which was shown using a GST pulldown. The proteins used in the experiment are made to mimic those in SMA patients so the interactions in a malfunctioning system could be identified. This involved mutating the SMN proteins so they included a valine where Asp44 is supposed to be. It is known that Gemin-2 contains Arg213 which interacts with Asp44 on the SMN protein. In patients with SMA, the genetic mutation causes a valine to replace Asp44 and a disruption in the ionic bond holding SMN and Gemin-2 together occurs. Gemin-2 was tagged with glutathione-s-transferase (GST) which acts like a handle. GST binds to beads that have a coat of glutathione on them. The mixture is then put into a centrifuge where the bead that is attached to GST and Gemin-2 will sink to the bottom. If Gemin-2 interacts with any other proteins in the mixture that too will be pulled down to the bottom as well. A gel can then be run to determine what interactions took place. If Gemin-2 did not bind to SMN it will travel farther in the gel than a complex were Gemin-2 bound to SMN.
It was found that in patients with SMA, the SMN proteins did not get pulled down to the bottom of the tube and therefore did not interact well with Gemin-2. A reciprocal experiment was completed where SMN was tagged and Arg213 was mutated to prove the same concept. Any disruption in the ionic bond between Asp44 and Arg213 will not allow SMN to bind effectively to Gemin-2. Because of this, the entire complex can no longer produce enough snRNPs to effectively splice RNA. The data set in the figure below, labeled "Total", shows that Gemin-2 and SMN were present in the tube before the experiment. The following data set shows that SMN was not pulled down with Gemin-2 in SMA patients.. The third and fourth data sets detail the results of the reciprocal experiment using SMN.

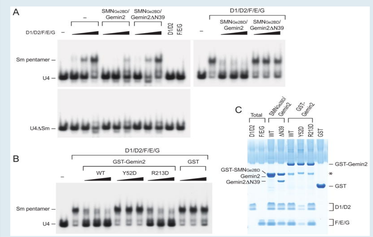


Figure Three
These figures are from experiments preformed by Gideon Dreyfuss and other researchers at the University of Pennsylvania School of Medicine. It was found that the N terminus of Gemin-2 plays a large role in the completion of snRNPs. Amino acid interactions were also found to be very important and it is clearly shown that the mutation from Asp44 to valine is the source of SMA.

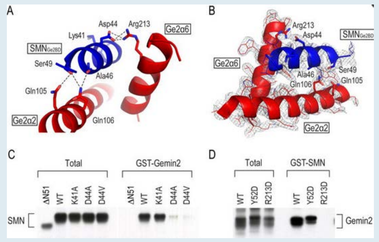


Figure Four
The ionic bond between Asp44 and Arg213 proved to be vital to the functioning of snRNPs. These two amino acids hold together the SMN complex, specifically Gemin-2 and the SMN protein, that makes snRNPs and without a strong bond not enough of these splicing machines can be made.

Conclusion

SMA is a devastating disease that can clearly trace its origins back to splicing. A small mutation or deletion of the SMN1 changes the amino acid sequence and disrupts the ionic bond that is vital for holding together the machinery that produces snRNPs. Because the SMN complex cannot produce enough snRNPs, cells are defective when splicing introns out of the genetic sequences. There is still no known reason why this leads to the degradation of motor neurons in the spine, but the effects of incorrect splicing on a molecular level can be still be seen in the big picture. Currently, scientists know that the splicing process begins to malfunction with a mutation in the SMN, causing improper interactions between the binding of Arg213 and Asp44, but scientists have yet to determine what causes the actual missense mutation. Another big issue with which scientists are grappling is why this condition is localized to motor neurons of the spine. Also, scientists have not developed a method to perfectly treat this disorder. Currently, there is one treatment that works inconsistently by manipulating the backup genes a patient possesses. Humans have multiple copies of the backup gene for SMN1, but these genes do not produce enough SMN protein to be sufficient. There is speculation that these backup genes could be the key to a cure in the future, especially with treatments like gene therapy on the horizon in mainstream medicine. However, there is still hope for the thousands of children diagnosed with this disorder as science continues to advance current treatments and innovate new cures.

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