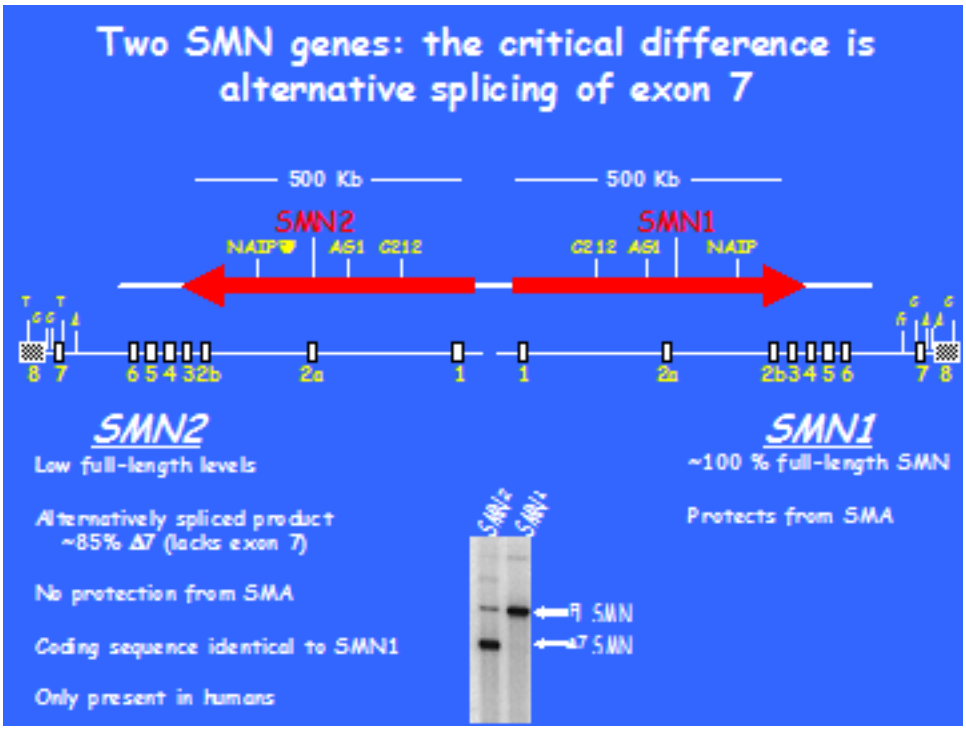
Research Statement

Molecular basis of spinal muscular atrophy; RNA processing; gene therapy.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder that is the leading genetic cause of infantile death. SMA is the most common inherited motor neuron disease and occurs in approximately 1:6,000 live births. The gene responsible for SMA is called survival motor neuron-1 (SMN1). Interestingly, a human-specific copy gene is present on the same region of chromosome 5q called SMN2. SMN2 is nearly identical to SMN1, however, mutations in SMN2 have no clinical consequence if SMN1 is retained. The reason why SMN2 cannot prevent disease development in the absence of SMN1 is that the majority of SMN2-derived transcripts are alternatively spliced, resulting in a truncated protein that lacks the 16 amino acids encoded by SMN exon 7 (normally the last coding exon). A single non-polymorphic nucleotide difference (C6T) between SMN1 and SMN2 is responsible for the alternative splicing of the SMN transcripts, however, this is a silent mutation that does not alter the overlapping protein coding capacity of SMN2. Numerous studies have shown that the SMN2-derived protein product (called SMN D 7) is unstable and dysfunctional, further demonstrating the critical nature of the SMN exon 7 splice site decision.

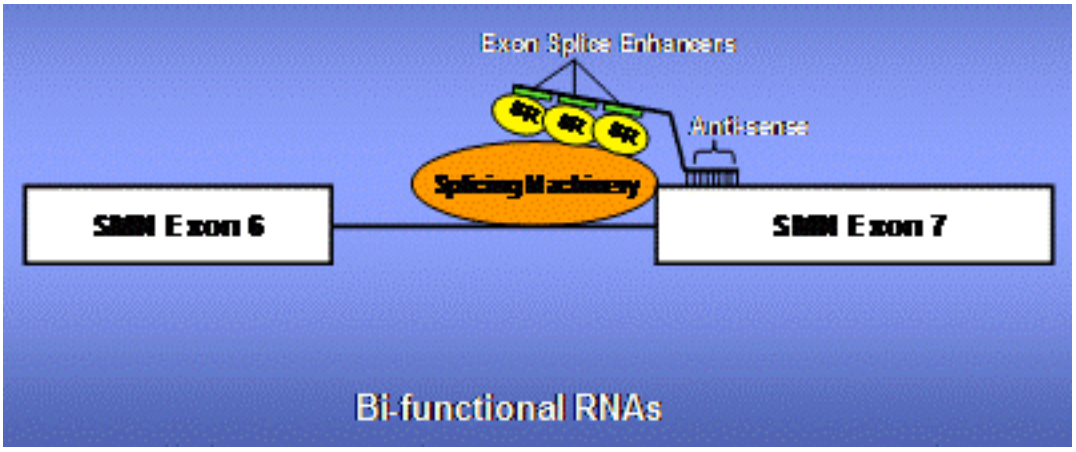


SMA is an extremely intriguing target for therapeutic intervention for a number of reasons: 1) While SMA presents in a broad clinical spectrum, a single gene is responsible for all clinical forms of the disease (severe; intermediate; mild); 2) Loss of SMN1andSMN2 is lethal, therefore essentially all SMA patients typically retain one or more copies of SMN2; 3) SMN2encodes a fully functional SMN protein. Therefore, by identifying molecules that stimulate full-length SMN expression from the SMN2 gene, these molecules could lead to the development of effective therapies for a broad range of SMA patient populations.

Several ongoing projects in the lab include:

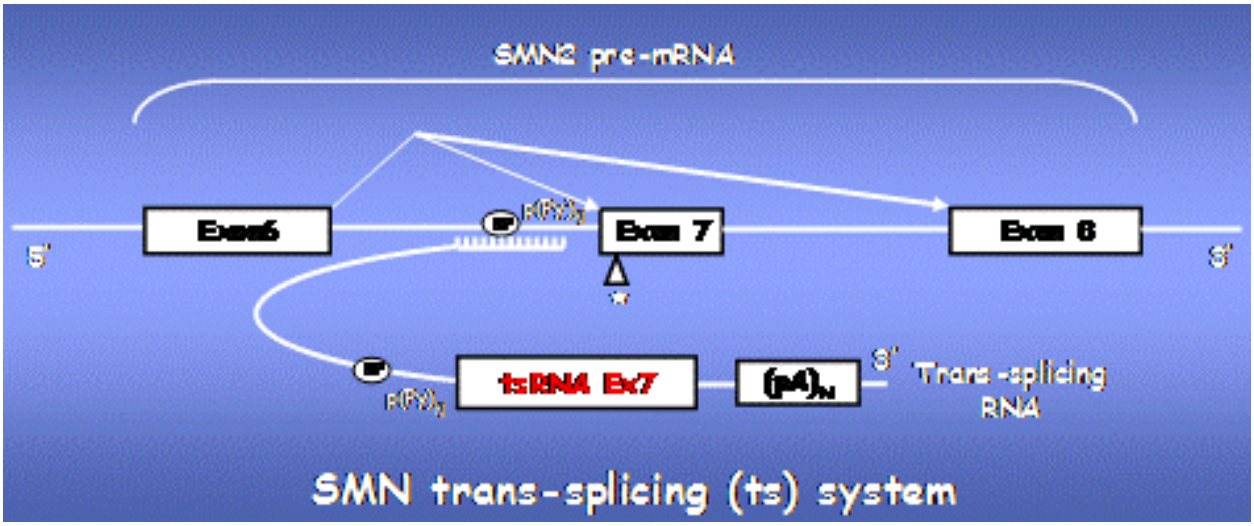
Bi-functional RNAs delivered via a gene therapy vector

To take advantage of the unique SMA genetic context, we are developing short RNAs that modulate SMN2 pre-mRNA splicing. Bi-functional RNAs derive their name due to the presence of two distinct domains: an RNA sequence that is complimentary to a specific cellular RNA (e.g. SMN); and an untethered RNA segment that serves as a sequence-specific binding platform for cellular splicing factors, such as SR and SR-like proteins. A large number of RNAs have been screened in cellular assays. From this work, a subset of lead candidate RNAs has been identified and will further delineate the most efficient RNAs that modulate SMN2 splicing patterns. The top candidate RNAs will then be expressed via a viral vector with and without the co-expression of a neurotrophic factors in a murine model of SMA. These experiments will determine whether a post-natal increase of SMN is sufficient or whether a soluble neurotrophic factor can aid in motor neuron survival. These experiments will also determine whether the RNAs can modulate SMN2 in an organism and whether this expected increase in full-length SMN2 expression lessens the SMA phenotype in transgenic mice.



Trans-splicing delivered via a gene therapy vector

Recently a therapeutic approach has been developed referred to as trans-splicing. Conceptually, this strategy relies upon pre-mRNA splicing occurring between two separate molecules: 1) the endogenous target RNA and 2) the therapeutic RNA that provides the correct RNA sequence via a trans-splicing event. SMN trans-splicing RNAs were initially examined and expressed from a plasmid-backbone and shown to re-direct splicing from a SMN2 mini-gene as well as from endogenous transcripts. Subsequently, recombinant adeno-associated viral vectors were developed that expressed and delivered trans-splicing RNAs to SMA patient fibroblasts. In the severe SMA patient fibroblasts, SMN2 splicing was redirected via trans-splicing to produce increased levels of full-length SMN mRNA and total SMN protein levels. Finally, snRNP assembly, a critical function of SMN, was restored to SMN-deficient SMA fibroblasts following treatment with the trans-splicing vector. Together these results demonstrate that the alternatively spliced SMN2 exon 7 is a tractable target for replacement by trans-splicing.



SMN-inducing compounds

Previously, we have shown that for some functions, heterologous sequences can compensate for the exon 7 peptide, suggesting that the SMN C-terminus functions non-specifically. Consistent with this hypothesis, we have identified novel aminoglycosides that can induce SMN protein levels in patient fibroblasts. This hypothesis was supported, in part, by a novel fluorescent SMN read-through assay. Interestingly, however, through the development of a

SMN exon 7-specific antibody, results suggested that levels of normal full-length SMN may also be elevated by aminoglycoside treatment. These results d

emonstrate that compounds that promote read-through may provide an alternative platform

for the discovery of compounds that induce SMN protein levels.