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Fixing what's lost in translation

By Eric Bender | Jun 01, 2015

Spinal muscular atrophy (SMA) is a heartbreaker, but it's not exactly a mystery.

The motor neuron disease is the leading genetic cause of mortality in children. More than half of infants born with SMA die before they reach their second birthday. There is no cure.

But scientists have long known the underlying genetic problem: mutations to the *SMN1* gene, which produces the survival motor neuron (SMN) protein. Without enough SMN, motor neurons wither and die. Evolution has a backup plan in the *SMN2* gene, a very close copy that also generates the critical protein. The backup gene, however, does a poor job of cranking out SMN because of a minor difference in its sequence that causes issues along the way to protein assembly.

Researchers at the Novartis Institutes for BioMedical Research (NIBR) report a potential solution online in *Nature Chemical Biology* on June 1. They've identified a molecule that increases production of SMN in cell and animal models. They've also demonstrated how the compound—which is being tested in a clinical trial in Europe — might work.

"Our compound can potentially boost SMN production by coaxing complex cellular machinery known as the spliceosome to help out," says Susanne Swalley, who is a co-first author on the paper and an investigator in Developmental & Molecular Pathways at NIBR.

To understand what the team did, let's take a closer look at the path to protein assembly.

The splice of life

DNA is made up of regions of exons (which act as templates for proteins) and introns (which don't). Initially, both exons and introns are transcribed into RNA. The RNA is then sliced and spliced, to bring together the exons and only the exons, before it is translated into protein. This slicing and splicing is performed by the cell's "spliceosome," which reassembles RNA in preparation for creating protein. With *SMN2*, the process usually misses a key chunk known as exon 7, so relatively few full-length SMN proteins are built—enough to support the development of an embryo, but not enough to keep a child healthy.

The Novartis researchers were determined to identify compounds that could potentially boost production of full-length SMN. The project launched with high-throughput screening of 1.4 million compounds on a neuron cell line, with two fluorescent reporters for *SMN2* splicing—one for full-length SMN and the other for SMN minus exon 7.

"We designed reporters to pull out needles from the haystack," explains Rajeev Sivasankaran, last author on the paper and a senior investigator in NIBR's Neuroscience group. "We identified some promising compounds as a result."

Following up on a small number of hits led to an early compound that showed some promise in a mouse model of severe SMA. A multi-year effort—led by Sivasankaran and chemist Natalie Dales—focused on optimizing drug-like properties, SMN elevation and efficacy in severe SMA mice, finally leading to the

identification of LMI070 (NVS-SM1), the small molecule that's currently being tested in a clinical trial of infants with the most severe form of the disease.

Finding the ties that bind

Exactly how does LMI070 work? The team suspected that it was influencing the spliceosome. Global RNA sequencing showed that LMI070 modulates only a very small set of splicing events, thus reducing worries about its selectivity and its potential side effects.

But how could it be operating selectively? The spliceosome is involved in stitching together templates for thousands of proteins. Why doesn't LMI070 affect these other templates?

The scientists' usual tools to identify a mechanism of action "didn't give us real actionable hypotheses that would explain the selectivity," says Swalley. "Everything we tried at the beginning failed."

Fortunately, co-first authors James Palacino and Cheng Song came up with a solution. They reasoned that there must be something special about the sequence of *SMN2* that explains the selectivity. So they took an unrelated gene on which LMI070 had no effect and added back bits of SMN2 to this gene to engineer a series of SMN2-like genes, each with a slightly different sequence. Then they inserted each SMN2-like gene into neuronal cells from mice to see if LMI070 worked on it. Using this clever strategy, they were able to pinpoint the specific SMN2 DNA sequence key to LMI070's activity.

The team zoomed in on a section of the *SMN2* gene at the edge of exon 7. It's an area that interacts with the splicing machinery—specifically, with one of the spliceosome's five main complexes, known as the U1 small nuclear ribonuclear proteins (snRNP). The team then discovered that LMI070 works by stabilizing the binding of *SMN2* with the U1 snRNP at this precise location, thus creating more RNA that is correctly spliced to act as a template for full-length SMN protein.

Finally, the investigators could describe the mechanism of action. And handily enough, a crystallography model of the U1 snRNP was published in January that is consistent with their hypothesis.

"I'm still amazed that we were able to figure out this very complex mechanism," says Swalley. "And if the compound does what we hope it does in patients, it has the potential to have a profound impact."

Lessons learned also may aid in treating other genetic diseases where splicing is implicated, among them some forms of autism, beta thalassemia blood disorders, certain kinds of colorectal cancer, the rare muscle illness myotonic dystrophy, and the aging disease progeria.

"We're excited to see our compound advance into clinical testing for a major unmet medical need and believe this novel mechanism paves the way for identifying selective splicing modulators for other diseases," says Sivasankaran.
