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A New Finding Clarifies the Cause of Spinal Muscular Atrophy

Howard Hughes Medical Institute (HHMI) researchers have made a surprising discovery about the molecular basis underlying spinal muscular atrophy (SMA), an often fatal neurodegenerative disease and the most common genetic cause of childhood mortality. The findings suggest that there may be a way to promote survival of neurons by helping a beneficial protein linger a little longer inside nerve cells.

Patients with SMA gradually lose the motor neurons in the spine that control most of their muscles. Researchers have known since the 1990s that the disease is nearly always linked to the absence or disruption of a gene known as *SMN1* (*Survival of Motor Neuron 1*). A nearby gene, *SMN2*, is virtually identical to *SMN1*, and in principle could produce enough SMN protein to keep neurons healthy -- yet somehow fails to do so.

In the March 2010, issue of the journal *Genes & Development*, HHMI investigator Gideon Dreyfuss and Sungchan Cho, a postdoctoral researcher in his lab at the University of Pennsylvania School of Medicine, report on their work solving this mystery.

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- Gideon Dreyfuss

Dreyfuss and Cho found that most of the SMN protein produced from *SMN2* is flagged for rapid degradation by a cellular waste-disposal system. Thus, the protein is cleared before it accumulates sufficiently to sustain the health of motor neurons. Blocking this degradation signal could therefore, in theory, be a way to treat SMA, Dreyfuss says.

Dreyfuss first identified the SMN protein in 1995. The subsequent discovery of a link between SMN and SMA led him to focus a considerable amount of his lab's work on the disease. In recent years, one aspect of that work has

been concerned with uncovering the function of the somewhat mysterious *SMN2* gene.

The *SMN2* gene differs from *SMN1* by a single letter of DNA, or nucleotide. This slight change appears to confound the cellular systems that turn gene sequences into RNA-based templates for the production of proteins. As a result, about 80 percent of the SMN protein copies produced from *SMN2* are missing a segment encoded by part of the gene known as exon 7.

This shorter version of SMN protein, termed SMN Δ 7, is almost undetectable in the cells of patients with SMA. But the shorter protein does seem to work: SMA patients who have more copies of the *SMN2* gene have milder signs of disease, and animal studies also suggest that increased levels of SMN Δ 7 can be beneficial. This suggests to scientists that if the level of SMN Δ 7 could be boosted therapeutically, patients might benefit.

To find out why SMN Δ 7 levels are so low in patients' cells, Cho and Dreyfuss created cells that produce SMN Δ 7 that is linked to luciferase, a light-producing enzyme found in the tails of fireflies. After shutting down new protein synthesis, they could observe how quickly the existing SMN Δ 7 was cleared from cells by observing the rate at which luciferase's glow faded.

Using this system, Cho and Dreyfuss confirmed that SMN Δ 7 disappeared much faster than normal-length SMN. The researchers then tinkered with SMN Δ 7, deleting certain segments at the malformed end to see whether this could restore the protein's stability.

They found that the improper junction between the segments that normally flank exon 7 had, by chance, created a signal that caused the entire protein to be degraded quickly. Removing this signal from SMN Δ 7 restored the protein to near-normal stability.

How does this signal cause the mutant protein to disappear so quickly? Cho and Dreyfuss found strong evidence implicating the proteasome system, a set of protein-crunching machines that roam through cells, destroying malformed or otherwise unwanted proteins. By suppressing proteasome activity, they restored SMN Δ 7 to normal stability, whereas suppressing other protein-clearance mechanisms had no effect.

"So ultimately SMN Δ 7 meets a proteasome and that is where it gets degraded," said Dreyfuss.

Proteasomes normally degrade only proteins that have been marked for destruction with special tagging molecules. These tags, in turn, bind to target proteins only after detecting certain molecular signatures they interpret as degradation signals, or degrons. "A degron is like a flag that says 'take me out,'" said Dreyfuss.

In this case, the experiments showed that the mutated end of SMN Δ 7 happens to form a degron. “Thus we found the cause of the extreme instability of SMN Δ 7,” Dreyfuss said. “That’s important, because it wasn’t clear why this protein simply vanishes.”

Further molecular tinkering by Cho and Dreyfuss revealed that, surprisingly, changing a single amino acid removed the degradation signal, allowing SMN Δ 7 levels to rise. This more stable version of SMN Δ 7 was enough to keep alive cultured cells, which quickly die without SMN.

“We don’t know yet whether the same approach will work in mammalian organisms,” said Dreyfuss. “But there are other reasons to believe that SMN Δ 7 contributes a similar function to that of normal-length SMN.”

Dreyfuss and his colleagues want to find out more about the cellular mechanisms that trigger SMN Δ 7 destruction. But they are already thinking about the development of therapies based on their findings. The fact that a single amino-acid substitution stabilizes SMN Δ 7 is encouraging, Dreyfuss says, suggesting that a drug compound might produce a similar effect.

Dreyfuss’s lab has been working on very broad drug screening efforts, including a large-scale collaboration with the pharmaceutical company Merck. “With these results we can develop more targeted screens for compounds that stabilize SMN Δ 7,” Dreyfuss said, “and we are pursuing this vigorously.”