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Vitamin Deficiency May Worsen Motor Neuron Disease

In the process of discovering a function for a common modification of proteins in the cell, researchers have found evidence that suggests that insufficient amounts of folic acid and vitamin B12 in the diet may exacerbate spinal muscular atrophy (SMA), a genetic disease that attacks motor neurons.

The scientists are planning to collaborate with clinicians to explore whether these vitamins might ameliorate the severity of symptoms in some SMA patients.

In a research article published in the May 2001 issue of the journal *Molecular Cell*, Howard Hughes Medical Institute investigator Gideon Dreyfuss and colleagues at the University of Pennsylvania School of Medicine report new information about how the protein, "survival of motor neurons" (SMN), which is reduced or defective in people with SMA, attaches to other proteins with which it interacts.

"Insufficiency of folate and B12 in SMA patients is a real possibility and this could lead to under-methylation of proteins, even if only slightly. Given this possibility, it would seem prudent for these patients, in consultation with their physicians, to ensure that their diet includes the recommended daily requirement of these vitamins."

- Gideon Dreyfuss

"SMN is a sort of master builder or chaperone that helps assemble many large RNA-protein machines (RNPs) in the cell," said Dreyfuss. "In particular, it appears to help construct complicated molecular machines that are critical for the production of messenger RNA." Messenger RNA plays an

essential role in ensuring that the information contained in DNA is properly translated into functional proteins.

SMA is the most common genetic cause of infant mortality, affecting about one in 6,000 newborns. The disease causes progressive muscle weakness, wasting, or atrophy as motor neurons degenerate.

The severity of the disease ranges from milder forms, in which people can live into adulthood, to more severe forms that cause death a few months after birth.

SMA is caused by deletions of one of the two genes that code for the SMN protein. Deletion of the gene reduces the level of SMN protein, which causes damage to the nerve cells that serve major muscle groups.

Loss of nerve stimulation causes muscles to atrophy and can result paralysis. In their studies, Dreyfuss and his colleagues probed how SMN binds to its multiple target proteins in the cell. Using biochemical methods, they determined that SMN binds to regions of target proteins that are rich in the amino acids arginine and glycine. "When we tested binding of SMN to these proteins directly *in vitro*, however, we found a surprisingly low affinity," said Dreyfuss. "We then began to suspect that something else must influence the binding, because when we isolated the target proteins from cells, SMN bound to them avidly."

To solve the mystery, Dreyfuss and his colleagues used the long-known fact that the arginines contained in many proteins are modified by the attachment of two molecules called methyl groups. "Dimethylation of arginines is a fairly common modification of proteins, especially RNA-binding proteins, that had first been reported more than thirty years ago," said Dreyfuss. "But the function of that modification was not known."

By using synthetic peptides with (or without) the methylated arginines as found in the cell, the researchers established that SMN did, indeed, bind tightly to its target proteins only when the arginines on those target proteins were dimethylated. "That alteration makes the modified protein interact with another protein -- in this particular case with SMN -- with much higher avidity," Dreyfuss said. The alteration of the arginines quite likely changes the shape of the protein's surface, making it fit SMN more snugly, he explained.

The finding -- which solves a three-decade-old scientific mystery -- provides a new research pathway for studying how proteins attach to one another and might also have clinical implications for people who have SMA, said Dreyfuss.

"These SMN target proteins obtain their methyl groups from a methyl donor called, *S*-adenosylmethionine, which itself depends on folate and vitamin B12

as part of its metabolic pathway," said Dreyfuss. Humans cannot produce or store folate and B12, so they must obtain those vitamins through their diet. "The thought is that SMA patients, who are already compromised in their levels of SMN, might be more severely affected if they are also suboptimal in their levels of protein methylation," said Dreyfuss.

"Insufficiency of folate and B12 in SMA patients is a real possibility and this could lead to under-methylation of proteins, even if only slightly," he said. "Given this possibility, it would seem prudent for these patients, in consultation with their physicians, to ensure that their diet includes the recommended daily requirement of these vitamins."

Dreyfuss is planning to collaborate with neurologists Thomas Crawford of The Johns Hopkins University School of Medicine and Richard Finkel of The Children's Hospital of Philadelphia to explore whether vitamin therapy might offer some relief to people with SMA.

Dreyfuss and his colleagues will also continue their studies of arginine methylation. They plan to begin looking for the enzyme that catalyzes attachment of methyl groups to the protein targets of SMN. Those studies, he said, could help reveal the regulatory pathway in a key cellular process that may extend to other proteins and other degenerative diseases of the nervous system.