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Toxic RNA Contributes to Neurodegeneration

In studies with fruitflies, researchers have discovered that a mutant RNA may be partially to blame for the neurodegeneration associated with spinocerebellar ataxia type 3 (SCA3). The toxic RNA may exacerbate the disease, which causes loss of muscle coordination and paralysis of the eye muscles and is known to be caused by the buildup of an aberrant protein inside neurons.

The scientists said their findings suggest that treatments that target the abnormal RNA might offer double benefits by alleviating both the protein as well as RNA toxicity.

"One possibility our findings suggest is that treatments that seek to alleviate Huntington's and other polyglutamine diseases by knocking down the RNA could offer two-pronged benefits."

— Nancy M. Bonini

Howard Hughes Medical Institute investigator Nancy M. Bonini and her colleagues published their findings April 30, 2008, in an advance online publication in the journal *Nature*. Co-authors include Lin-Bo Li, Zhenming Yu and Xiuyin Teng, who were all members of Bonini's laboratory at the University of Pennsylvania.

Over the last decade, Bonini and her colleagues have painstakingly recreated models of human neurodegenerative disease in the fruit fly. Their work involves introducing the gene for the human neurodegenerative disease into the fly, recreating the effects of the disease so that it can be studied in a highly manipulable system.

In the studies reported in *Nature*, the researchers used a strain of the fruitfly *Drosophila* that had a mutation in the gene that is altered in human patients with SCA3. This fruitfly strain exhibits neural degeneration and is a useful laboratory model for human SCA3.

SCA3 is one of a class of diseases known as polyglutamine repeat diseases. SCA3 and the other polyglutamine diseases, like Huntington's disease and SCA1, are caused by genetic mutations consisting of an abnormally long number of repeats of three nucleotides, also known as triplet repeats. The length of the genetic stutter of nucleotides can vary in each disease. For this set of diseases, the repeated nucleotide triplet is CAG, which encodes an amino acid called glutamine. The mutant version associated with human disease thus leads to a protein with an abnormally long glutamine string. The malformed protein is toxic to cells and causes neurological degeneration.

In SCA3 and similar diseases, the length of this glutamine string can grow as it is passed down from one generation to the next. This feature of the disease, called repeat instability, has important clinical implications because expansion of repeats causes the disease to arise earlier and with greater severity in successive generations of people who carry the mutation, Bonini explained.

When the genetic causes of polyglutamine diseases were first identified, researchers found that the aberrant proteins were toxic, but they did not look closely at whether the RNA could be toxic as well, Bonini said. To convert a gene into protein, cells begin by copying DNA into RNA. The RNA molecule serves as the template the cell uses to build a protein.

Bonini and her colleagues did not set out to use their fruitfly model of SCA3 to search for signs of RNA toxicity. Rather, they let the fly tell them in an unbiased way about genes that could improve or worsen symptoms of the disease. During these studies, the researchers discovered that activating the *muscleblind* (*mb1*) gene in the flies dramatically increased neural degeneration.

Further analyses suggested that the *mb1* gene exacerbated the disease by interacting with RNA. And when the researchers inserted the human version of the *mb1* gene into the fly, they saw that the neurodegeneration also worsened, providing a hint that *mb1* might also play a role in human polyglutamine diseases.

Intrigued by the possibility that the RNA itself was toxic, Bonini and her colleagues next molecularly interrupted the repetitive RNA sequence. Normally, the RNA has a long series of CAG repeats; the researchers interrupted the sequence so that it now had CAA and CAG repeats. This RNA sequence encodes the same mutant protein, but the RNA was not predicted to fold in the same toxic manner. When they introduced this interrupted RNA back into their fruitflies, significantly less neurodegeneration occurred, further indicating that the RNA itself might be toxic.

Then the researchers genetically engineered mutant flies that produced only the repetitive RNA but no aberrant protein. Those flies showed significant neural degeneration. That finding suggested that the RNA on its own contributes to the pathology, said Bonini.

She said the new results reveal that polyglutamine diseases like SCA3 and Huntington's disease may share the property of RNA toxicity with a different class of triplet repeat diseases, which includes myotonic dystrophy type 1 and fragile X-associated ataxia. Those diseases also arise from genetic mutations that produce a stuttering RNA. That RNA does not, however, produce an abnormal, toxic protein; the pathology of those diseases is thought to arise only from the toxicity of the RNA.

Although the new studies suggest that the two classes of triplet repeat diseases are caused in part by RNA toxicity, Bonini said their analyses indicate that the mechanism of RNA toxicity appears to be different for each class of disease. In future studies, the researchers will concentrate on determining the mechanisms of RNA toxicity in the polyglutamine disease fly model, she said.

Bonini said the new findings could offer the promise of more effective treatments for polyglutamine diseases. One possibility our findings suggest is that treatments that seek to alleviate Huntington's and other polyglutamine diseases by knocking down the RNA could offer two-pronged benefits, she said. They would not only reduce the level of the toxic protein, but also knock down the toxic RNA. Thus, such treatments might be more effective than those directed only at the protein, she said.

Ling-Bo Li is now a postdoc in a Hughes Lab at Utah (with Brenda Bass).