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Genetic mutation found to restore translational balance in mice

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In a biological quirk that promises to provide researchers with a new approach for studying and potentially treating Fragile X syndrome, scientists at the University of Massachusetts Medical School (UMMS) have shown that knocking out a gene important for messenger RNA (mRNA) translation in neurons restores memory deficits and reduces behavioral symptoms in a mouse model of a prevalent human neurological disease. These results, published today in *Nature Medicine*, suggest that the prime cause of the Fragile X syndrome may be a translational imbalance that results in elevated protein production in the brain. Restoration of this balance may be necessary for normal neurological function.

"Biology works in strange ways," said Joel Richter, PhD, professor of molecular medicine at UMMS and senior author on the study. "We corrected one genetic mutation with another, which in effect showed that two wrongs make a right. Mutations in each gene result in impaired brain function, but in our studies, we found that mutations in both genes result in normal brain function. This sounds counter-intuitive, but in this case that seems to be what has happened."

Fragile X syndrome, the most common form of inherited mental retardation and the most frequent single-gene cause of autism, is a genetic condition resulting from a CGG repeat expansion in the DNA sequence of the Fragile X (Fmr1) gene required for normal neurological development. People with Fragile X suffer from intellectual disability as well as behavioral and learning challenges. Depending on the length of

the CGG repeat, intellectual disabilities can range from mild to severe.

While scientists have identified the genetic mutation that causes Fragile X, on a molecular level they still don't know much about how the disease works or what precisely goes wrong in the brain as a result. What is known is that the Fmr1 gene codes for the Fragile X protein (FMRP). This protein probably has several functions throughout the neuron but its main activity is to repress the translation of as many as 1,000 different mRNAs. By doing this, FMRP controls synaptic plasticity and higher brain function. Mice without the Fragile X gene, for instance, have a 15 to 20 percent overall elevation in neural protein production. It is thought that the inability to repress mRNA translation and the resulting increase in neural proteins may somehow hamper normal synaptic function in patients with Fragile X. But because FMRP binds so many mRNAs, and some proteins become more elevated than others, parsing which mRNA or combination of mRNAs is responsible for Fragile X pathology is a daunting task.

From Frog Egg to Fragile X

For years, Dr. Richter had been studying how translation, the process in which cellular ribosomes create proteins, went from dormant to active in frog eggs. He discovered the key gene controlling this process, the RNA binding protein CPEB. In 1998, Richter found the CPEB protein in the rodent brain where it played an important role in regulating how synapses talk to each other. At this point, his work began to move from exploring the role of CPEB in the developmental biology of the frog to how the CPEB protein impacted learning and memory. A serendipitous research symposium with colleagues at Cold Spring Harbor got him thinking about CPEB and Fragile X syndrome.

"Here I was, an outsider, a molecular biologist who had worked for years with frog eggs, in the same room with neurobiologists and neurologists,

when they started talking about Fragile X syndrome and translational activity," said Richter. "It got me thinking that the CPEB protein might be a path to restoring the translational imbalance they were discussing."

Richter knew that CPEB stimulated translation and that FMRP repressed it. He also knew that animal models lacking the CPEB protein had memory deficits and that both proteins bound to many of the same mRNAs – the overlap may be as higher as 33 percent. The thought was that by taking away a protein that stimulated translation might counterbalance the loss of the repressor FMRP protein, thereby restoring translational homeostasis in the brain and normal neurological function.

"It was one of those kind of goofy 'what if' sort of things," said Richter.

To test his hypothesis, Richter developed a double knockout mouse model that lacked both the FMRP gene that caused Fragile X and the CPEB gene. When they began measuring for Fragile X pathologies what they found was almost too good to be true.

"We measured a host of factors, biochemical, morphological, electrophysiological and behavioral phenotypes," said Richter. "And we kept finding the same thing. By knocking out both the FMRP and CPEB genes we were able to restore levels of protein synthesis to normal and corrected the disease characteristics of the Fragile X mice, making them almost indistinguishable from wild type mice."

Most importantly, tests to evaluate short-term memory in the double knockout mice also showed normal results with no indications of Fragile X pathology. This suggested an experiment to test whether CPEB might be a potential therapeutic target for Fragile X to benefit patients. Richter and colleagues took adult Fragile X mice and injected a lentivirus that expresses a small RNA to knock down CPEB in the hippocampus, which is a brain region that is important for short-term memory. Subsequent

tests showed improved short-term memory in these mice, indicating that at least this one characteristic of Fragile X syndrome, which is generally thought to be a developmental disorder, can be reversed in adults.

"People with Fragile X make too much protein," said Richter. "By using CPEB to recalibrate the cellular machinery that makes protein we've shown that tamping down this process has a profoundly good impact on mouse models with Fragile X. It may be that a similar approach could be beneficial for kids with this disease."

The next step for Richter and colleagues is to determine which, of the more than 300 mRNAs that both CPEB and FMRP bind to, contribute to Fragile X syndrome and how. They'll also begin looking at small molecules and other avenues that, like the ablation of the CPEB protein, might be able to slow down the synthesis of protein. "There are several small molecules that we know affect the translational apparatus," Richter said. "Some cross the blood/brain barrier, some are toxic, and some are not. We'd like to investigate those."

"This is another, great example of how basic science translates to human disease," said Richter. "If we had started out looking at the human brain, not knowing about the CPEB protein and its role in translational activity, we wouldn't have had any idea where to start or what to look for. But because we started out in the frog, where things are much easier to see, and because more often than not these processes are conserved, we've learned something new and totally unexpected that may have a profound impact on human disease."

More information: Genetic and acute CPEB1 depletion ameliorate fragile X pathophysiology, DOI: 10.1038/nm.3353