

DECEMBER 01, 1999

## Understanding Key Protein in Fragile X Syndrome

Fragile X syndrome, the most common inherited form of mental retardation, results from a mutation that affects how genetic messages are ferried from the cell's nucleus to its protein manufacturing apparatus. The identification of three new molecules integral to the protein shuttling process has researchers bearing down on the cellular mechanisms that underlie fragile X syndrome, and perhaps other causes of mental retardation as well.

For a condition with such a delicate-sounding name, fragile X syndrome can cause a number of powerful effects, ranging from learning disability and hyperactivity to severe mental retardation. Mutations in the *FMR1* gene produce the disorder, and Stephen Warren, a Howard Hughes Medical Institute (HHMI) investigator at Emory University, has been studying this gene and the protein it produces, called FMRP, since he and his colleagues first identified them in 1991.

"When we first identified FMRP, it didn't look like any other protein that had been identified, so we really didn't know what it did. But by slowly chipping away over the years, we and others have learned a lot about it," said Warren.

For example, Warren's group has determined that FMRP binds to messenger RNA (mRNA) molecules and forms a complex called a RNP (ribonucleoprotein particle). They also know that FMRP shuttles between the cell's nucleus and cytoplasm, visiting ribosomes, where the genetic code for building proteins is translated. This evidence suggested to Warren and his colleagues that FMRP is somehow involved in the translation process.

To understand more about FMRP's function in the cell, Warren's group needed to identify the protein's partners in the RNP complex, a feat that had defied conventional protein-purifying techniques. Not to be thwarted, the researchers developed a set of antibodies that allowed them to isolate not only FMRP, but also messenger RNA (mRNA) and at least six other proteins from the complex. Warren, together with colleagues Stephanie Ceman and Victoria Brown, published these results in the December 1999 issue of the journal *Molecular and Cellular Biology*.

With these components in hand, the three researchers proceeded to identify three of the proteins in the complex. Two of the proteins, FXR1P and FXR2P, belong to the same family of proteins as FMRP.

A surprise awaited the HHMI team when it identified the third protein as nucleolin, a protein that scientists thought stays tucked away in the nucleus. Placing nucleolin in the RNP complex suggests that it wanders out to the cytoplasm and may help regulate translation.

Since the *Molecular and Cellular Biology* paper went to press, Warren's group has tentatively identified additional, more interesting, proteins in the RNP complex. One is similar to a yeast protein that helps unwind mRNA during translation. Because translation is known to be governed in part by changes in mRNA structure, this protein may also play an important role in regulating the process.

Identifying the proteins in the RNP complex is important, said Warren, because they may be involved in other types of mental retardation. "For most forms of mental retardation in humans, the genes have not been identified. Now that we're identifying some of these proteins, the next step will be to start screening patients to see if they have the associated genes."

Warren's group is also starting to study the mRNA from the RNP complex. "Isolating the RNA that's specifically interacting with the FMRP will give us a handle on the biggest question in this whole area of research what are the messages that are bound to it," said Warren, who expects to identify most of the messages that bind to FMRP within the next year. With the messages in hand, the researchers may finally get a handle on how mutant FMRP the root molecular cause of fragile X syndrome causes mental retardation.

Initially, Warren's group isolated the proteins from cultured connective tissue cells. While people with fragile X syndrome sometimes have connective tissue abnormalities, their mental deficits are the greatest concern, so an obvious question is whether the same proteins are found in the brain. By modifying their technique, the researchers were also able to isolate FMRP and the other proteins from mouse brains.

"Now we've begun to identify the messages from the brain," said Warren, "and doing that should give us substantial new insights into the syndrome."