

JULY 06, 2001

Evidence that Alzheimer's Protein Switches on Genes

Researchers have found the first evidence that indicates a cellular function for the protein that produces the brain-clogging amyloid plaque deposits that cause Alzheimer's disease. The scientists found that a fragment of the amyloid β -protein precursor that is snipped off and remains inside cells can switch on genes. By studying how these wayward protein fragments affect gene activity, researchers may learn more about the origins of some forms of Alzheimer's disease.

Howard Hughes Medical Institute investigator Thomas C. Südhof and colleague Xinwei Cao at the University of Texas Southwestern Medical Center published their findings in the July 6, 2001, issue of the journal *Science*.

"One might theorize that a possible cause for sporadic Alzheimer's disease is misregulation of this type of gene expression, and that creation of amyloid plaque is a byproduct of this misregulation."

— **Thomas C. Südhof**

The scientists concentrated their efforts on searching for a function of the amyloid β -protein precursor (APP). APP is snipped apart by enzymes, in a reaction called proteolytic processing, to produce three protein fragments. Two fragments remain outside the cell and one stays inside. When APP is produced in excessive quantities, one of the cleaved segments that remains outside the cell, called the amyloid β -peptide, clumps together to form amyloid plaques that kill brain cells and may lead to the development of Alzheimer's disease.

"There is a vast literature describing the enzymes and mechanisms of proteolytic processing of APP and their role in Alzheimer's disease," said Südhof. "But as far as I am aware, there is no reported work on the potential physiological importance of that processing."

Südhof and Cao believed that the short "tail" segment of APP that is trapped in the interior of the cell could also contribute to Alzheimer's disease. The scientists theorized that this tail might switch on genes — a process called transcriptional activation — because it was similar to a *Drosophila* protein called Notch. The same type of enzyme that cleaves APP also snips apart Notch. When Notch is cleaved, a fragment of Notch is produced that activates gene transcription. In further support of their idea, APP and its molecular cousins APLP1 and APLP2 resemble cell-surface receptors whose breakdown appears to be triggered by an external chemical signal.

To explore the role of the APP tail, Südhof and Cao created a version of APP into which they inserted either of two DNA-binding proteins called Gal4 and LexA that switch on specific indicator genes. The Gal4 or LexA proteins were inserted into APP in such a way that they would hitchhike with the cleaved tail segment, and would activate transcription of specific genes if the tail segment were incorporated into a cell's DNA transcription machinery.

To their surprise, however, when the Gal4- or LexA-containing APP was inserted into cells alone, there was no detectable gene transcription. "That finding suggested to us either that APP has nothing to do with transcription or that we were missing another factor that promoted transcription," said Südhof.

In a series of experiments, Südhof and Cao incorporated into the cells a range of proteins known to bind to the APP tail. "That's when we struck gold," said Südhof. "We found that when we included the protein Fe65, we got as much as a several-thousand-fold increase in transcription."

The Fe65 protein, said Südhof, is an adaptor protein that aids the function of other proteins. When Fe65 was bound to a key segment of the tail, called a binding domain, the researchers could see that there was another binding domain on the tail that could bind another protein. In additional experiments, the scientists found that the protein Tip60 bound to second binding site on the tail of APP. Tip60 is a nuclear protein that incorporates itself into a large protein complex involved in DNA transcription. Test tube studies revealed that the APP tail, Fe65 and Tip60 formed a stable complex.

Südhof and Cao next attached Gal4 only to Tip60 and inserted that complex into cells with APP and Fe65. Those experiments showed clear evidence that gene transcription occurred. "In those particular experiments, there was no modification of APP or Fe65 — just Tip60," emphasized Südhof. "And Tip60 is known to be a nuclear protein."

The regulation of APP proteolytic processing is a central question that Südhof and his colleagues plan to address in future studies. "I believe that this process is normally regulated, and that this regulation in effect, determines how much amyloid-beta peptide is produced," said Südhof. "So, one might theorize that a possible cause for sporadic Alzheimer's disease, is misregulation of this type of gene expression, and that creation of amyloid plaque is a byproduct of this misregulation."