Family of protein fragments promises fresh view of Alzheimer's disease

Charlotte Schubert, *Science Writer*

Neuroscientist Joris de Wit was pretty sure he was on the right track as he homed in on a receptor for a potentially beneficial brain molecule. Known as sAPPalpha (soluble amyloid precursor protein alpha), the molecule is a seemingly benevolent sibling to amyloid beta, the peptide that forms clumps in the brains of people with Alzheimer's disease. De Wit's confidence grew when he received a call from his structural biologist collaborators. "Something is actually happening," they told him.

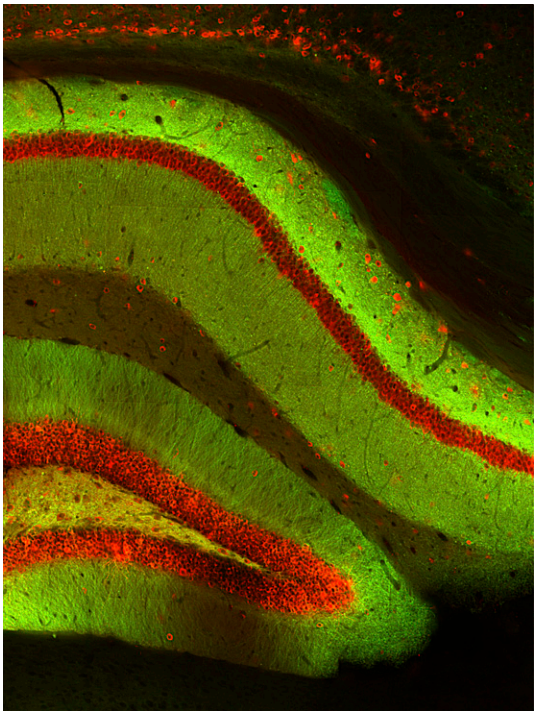
For months, the group had been stymied—the receptor motif they were trying to characterize was floppy and unstructured. Then they decided to add a short peptide corresponding to a region of sAPPalpha. The receptor motif and peptide zipped together, and a model of the structure emerged.

The resulting study, recently published in *Science* (1), shows that sAPPalpha binds to a receptor for the neurotransmitter glutamate, modulating the transmission of neural signals across synapses. The findings suggest that sAPPalpha can quell excess neurotransmission and thereby contribute to brain health. They also offer further clues of the part a special class of protein fragments plays in the development of Alzheimer's disease.

A hardy cadre of researchers is looking to understand the precise inner workings of amyloid precursor protein (APP) and its assorted fragments, including sAPPalpha—and what happens to those fragments when things go awry. Progress is slow and piecemeal in this potentially key subfield of Alzheimer's research—and has been for years.

Slowly, the picture is becoming clearer. One idea is that APP is a regulator of neurotransmission, explains Bart De Strooper, who co-led the study with de Wit at the VIB-KU Leuven Center for Brain & Disease Research in Leuven, Belgium. And different pieces of APP, notes De Strooper, appear to affect neurotransmission differently. A shift in the balance of their functions, as some fragments increase their activity and others lose influence, could contribute to the development of Alzheimer's disease.

The complexities of APP and its fragment progeny still often defy the efforts of researchers seeking to pinpoint functions and elusive drug targets. But the work of De Strooper, de Wit, and others is yielding fresh ideas in a field that many scientists say badly needs new approaches.



sAPPalpha (red), expressed here in mice, seems to have beneficial effects on the brain. In experiments in mouse models of Alzheimer's disease, sAPPalpha expression improves memory (dendritic neuron processes shown in green). Image credit: Heidelberg University Institute of Pharmacy and Molecular Biotechnology/Max Richter and Ulrike Müller.

Amyloid Revisited

Alzheimer's research has long been propelled by a central idea, dubbed the amyloid hypothesis. It holds that deposition of amyloid beta initiates a cascade of events that includes synapse dysfunction, inflammation, neuronal loss, and ultimately dementia.

Some of the strongest evidence for the hypothesis is from human genetic studies (2). Mutations affecting the proteases that chew up APP are associated with early-onset Alzheimer's disease as well as an increased

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build-up of amyloid beta. Protective mutations result in minimal amyloid accumulation.

But genetic data on fragments other than amyloid beta are sparse (3). Moreover, long-term human studies, including work on donated brains of deceased individuals with disease, have shown that not everyone who has amyloid buildup has dementia. The correlation is "not great," says Sally Hunter, who was involved in such studies in the lab of Carol Brayne at the University of Cambridge in Cambridge, United Kingdom (4). And then there are the disappointing clinical trial results. Over the past decades, dozens of trials targeting amyloid beta have been discontinued for lack of efficacy, side effects, or even for worsening cognitive symptoms. The latest example came in March, when Biogen, based in Cambridge, MA, halted two closely watched trials of an antibody designed to clear amyloid beta from the brain—the announcement sent its stock price plummeting.

Researchers are looking at the amyloid hypothesis with renewed scrutiny. Physician-researcher Michael Heneka, for example, still thinks that amyloid beta is a key player. But the more important question, he says, is "at which stage of the pathology it sets in and what is the consequence for its deposition." Seeking a fresh approach, Heneka, director of the Department of Neurodegenerative Diseases and Gerentophysiology at the University of Bonn in Germany, is studying the immune system, which his group has found plays a role in amplifying amyloid beta deposition in the brain (5).

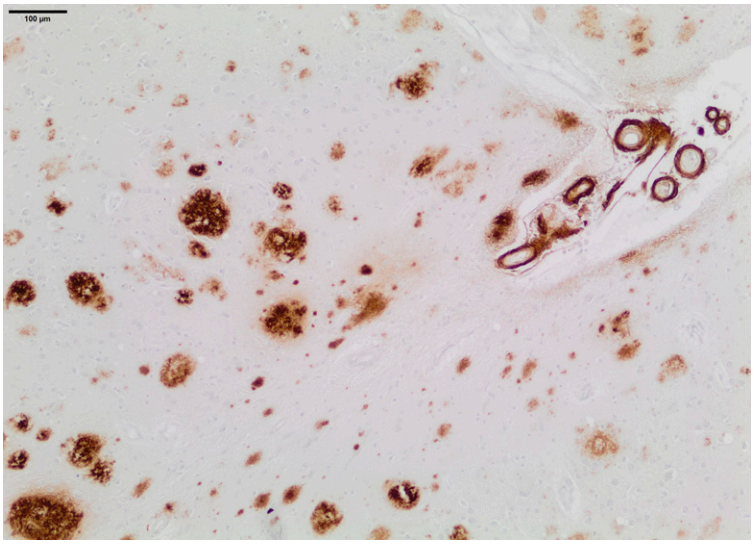
As researchers explore newer ideas about Alzheimer's disease, such as the role of inflammation and immunity, some emphasize getting a firmer grasp on how APP and its many cleavage products operate. One reason is to better understand experimental drugs that target amyloid beta but that also may affect other APP fragments, says Heneka. For example, what side effects might arise? That question has become more urgent given recent clinical trial designs that involve subjects at earlier stages of the disease, says Heneka, who's also a consultant for Boston-based IFM Therapeutics and Alector in South San Francisco. "I would want to absolutely be sure there are no long-term effects," he adds.

Hui Zheng, who has been studying APP for more than a decade, notes that several Alzheimer's therapies in the pipeline modulate the enzymes that chop up APP. That makes understanding APP's various functions highly relevant for drug development. "You are changing all the other APP products," Zheng notes. "What do those do?"

Fragment Physiology

Zheng believed so strongly in the importance of understanding APP's basic biology that she left a job in the pharmaceutical industry in 1999 to start a lab at Baylor College of Medicine in Houston and explore more fundamental questions. "Amyloid is not just an unfortunate event in evolution," she says. "People have to understand it."

So far, researchers have gleaned that APP and its molecular spawn are multitaskers, sometimes working at cross-purposes. Whole APP seems to have roles in cell adhesion and the outgrowth of neural extensions.



Several types of amyloid beta deposition (red) are visible in the human brain, including in the vasculature (upper right). One of the many outstanding questions in Alzheimer's research is why amyloid is found in various forms. Image credit: Sally Hunter.

Intracellular fragments can affect gene expression to promote cell death or cell differentiation. Some extracellular fragments have only recently been discovered, and others have barely been studied (6).

Zheng and her colleagues recently identified a potential receptor for the whole APP molecule. Called Slit, it's involved in axon guidance (7). Her findings dovetail with other research suggesting that APP can help guide where neuronal extensions grow and make connections. But the work was highly challenging. "APP is a very tough molecule," she says. For instance, it tends to stick to a lot of other things in the cell. So, despite many proposed candidates, "it is very difficult to know what is a bona fide receptor."

De Wit and his colleagues, including first author Heather Rice, faced similar challenges in pinpointing a receptor for sAPPalpha. They had identified a potential receptor from nerve terminal extracts, $GABA_BR1a$, a subunit of the receptor for the neurotransmitter GABA (γ-aminobutyric acid). But they couldn't be sure it wasn't just another sticky interaction. De Wit's opportune call from the structural biologists helped build the case that they had uncovered a genuine receptor.

The researchers also performed a series of functional studies examining how sAPPalpha affects neurotransmission in rodent brains. For instance, de Wit and collaborators analyzed neuronal activity in live mouse brains using a technique called two-photon calcium imaging and showed that applying a peptide corresponding to a region of sAPPalpha suppressed neuronal activity (1). The researchers' findings also suggested two other APP fragments may act similarly.

De Strooper, who now directs the new UK Dementia Research Institute based at University College London in London, says evidence that these APP fragments can quiet neural activity and that they operate by binding presynaptically to $GABA_BR1a$ fits with a scenario in which other parts of APP modulate the activity of

amyloid beta. Noting that there is also evidence amyloid beta can increase neuronal excitation, De Strooper speculates that sAPPalpha could counteract the effect of amyloid beta, perhaps by blunting neuronal excitation.

And there could be some implications for therapy design, though it remains early days. People developing Alzheimer's disease may undergo a period of excess neural activity. "Before you see cognitive symptoms you see something called hypersynchronicity," explains Martin Korte, an Alzheimer's researcher at the Technische Universität Braunschweig in Braunschweig, Germany. "The neurons in the brain become too active." (8) Korte speculates that bumping up the action of sAPPalpha, for instance by administering a short peptide, could dampen some of this hyperexcitability and potentially fend off Alzheimer's disease—an idea that could be tested in Alzheimer's animal models. Meanwhile, several other conditions, such as epilepsy and schizophrenia, involve $GABA_BR1a$ and might also respond to its modulation.

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>
> —Sally Hunter

Ulrike Müller, a professor at the Institute of Pharmacy and Molecular Biotechnology at the University of Heidelberg in Germany, and her colleagues, including Korte, have found that overexpressing sAPPalpha in the brains of a mouse model of Alzheimer's disease mitigated neuronal defects and improved memory (9). More recently, Müller's group has shown that sAPPalpha may operate through a specific acetylcholine receptor to affect synaptic function, plasticity, and memory in mice (10). "It's beneficial, it's good for the brain," says Müller.

Back to Basics

Broadening the focus of investigation beyond amyloid beta has prompted a new look at the mechanisms of Alzheimer's disease. In a widely cited review article, De Strooper and Eric Karran, now Vice President at the AbbVie Foundational Neuroscience Center in Cambridge, MA, propose that the brain can compensate for amyloid beta build-up and accompanying effects for many years (11). "It is only at the late stage that you start to get this collapse which we call dementia," explains De Strooper. Eventually, as cellular homeostasis breaks down, the brain tips into disease.

Other researchers have put forward related ideas, including Hunter and Brayne (12, 13). APP impinges on a lot of cellular processes, says Hunter, "and then those fragments that it releases go back to affect those processes, so you get this iterative cycle." This view emerged from years of looking at the pathology of human brains with dementia, Hunter adds. "I was instantly hit by how complex it was. And I was really hit by the idea that you need a complex model to fit the natural system."

Ultimately, many researchers predict that Alzheimer's disease may yield to combination therapies that hit multiple systems. There are several potential targets, including the various APP fragments. To strike the right balance, the field would benefit from "people with new ideas that have not previously done Alzheimer's research," says de Wit, whose main remit is synapse biology. Devising effective therapies will require greater knowledge of the biological mechanisms behind Alzheimer's disease, including more insight into APP and its fragments. De Strooper puts it succinctly: "We need to know much more."

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