

NEWS

Atomic close-up of brain proteins hints at diversity of autism

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Perfect partners: When mixed, the autism-related protein SHANK (center), and another brain protein, Homer (left), form a sturdy mesh (right) that neither protein can form when incubated alone.

Scientists have unveiled the complete structure of an interwoven complex of two types of brain proteins necessary for forming and maintaining connections between neurons.

One of the proteins belongs to the SHANK family, which has been implicated in several genetic studies of people with autism¹. The new molecular map of the complex may offer clues about how nerve connections go awry in the disorder, say the researchers.

As people adapt to new experiences, the most active synapses — junctions between neurons — grow larger and stronger, whereas the least-used connections get smaller and weaker. Synapses change their strength with help from the post-synaptic density (PSD), a mix of hundreds of signaling molecules that cluster on the receiving end of the synapse. Some studies suggest that the PSD grows during learning and memory tasks².

Interested in the molecular framework behind synaptic changes, researchers isolated from rat brain tissue two proteins — Homer and SHANK — which are found in abundance in the PSD. Using a

technique called X-ray crystallography, the team identified the precise arrangement of the atoms within each of the proteins. The work was published in the April issue of *Cell*³.

"Homer and SHANK make synapses enlarged, so I thought they may have some structural role, and that's why I started to study them," says Mariko Hayashi, assistant professor of pharmacology at Keio University in Tokyo. She conducted the work as a postdoctoral associate at the Massachusetts Institute of Technology.

After mixing the proteins and incubating them overnight, the scientists determined that the molecules tightly interlock like a chain-link fence, producing a sturdy mesh that neither protein can form when incubated alone.

What's more, the team found that two other common synaptic molecules in the PSD — PSD-95 and GKAP — can bind to the Homer-SHANK mesh without disrupting its structure. This suggests that the complex forms a supportive scaffold that many other PSD proteins can latch onto, they say.

SHANK is spherical, whereas Homer is a long and dumbbell-shaped, with each end holding two sites for binding to other molecules.

"Homer gives flexibility for large proteins to bind," says Hayashi. "This fact couldn't be proved without the [X-ray crystallography]. It was very exciting to see that structure on the screen."

Decoding SHANK:

The new findings may help answer some questions posed by previous genetic studies of synapse protein mutations in people with autism.

In 2007, for example, researchers led by **Thomas Bourgeron** found variations in the SHANK3 gene in 3 out of 226 families with at least one child with autism. The gene codes for the SHANK3 synapse protein.

The specific SHANK3 variations identified in Bourgeron's study were surprisingly diverse. A boy in one family is missing a large portion of the gene, whereas two brothers in a second family have smaller deletions. In the third family, a girl with autism and severe language delay is missing one copy of SHANK3, whereas her brother, diagnosed with the milder Asperger syndrome, has three copies.

"The gene dosage of the SHANK3 gene is very puzzling," says Bourgeron, director of the Human Genetics and Cognitive Functions unit at the Institut Pasteur in Paris.

The new crystallography study focused on the SHANK1 protein, but might still explain some of the diversity found in Bourgeron's report. Slightly too much or too little of a SHANK gene could

drastically change the amount of protein produced, disrupting the intertwined structure of the PSD and, ultimately, the strength of the synapse.

"If it is a highly organized structure, then the protein dosage between Homer and SHANK must be perfect," Bourgeron says. "If you have a deletion of one dose of SHANK, the structure will be altered. If you have three copies of SHANK, again the complex may also be altered."

A 2006 study in *Science* found that SHANK3 forms highly organized molecular sheets in the PSD⁴. Similar to the new study, the authors proposed that these sheets form a scaffold upon which other synaptic proteins can bind.

By affixing proteins in this way, Homer and SHANK may help regulate synaptic plasticity, the cellular learning process whereby sensory stimuli strengthen or weaken synapses, Hayashi notes. Previous studies have linked autism-related disorders to abnormal **synaptic plasticity**.

Tagged mice:

Because there's no direct way to measure synaptic plasticity in humans, researchers say it will be particularly important to study the synaptic protein interactions between Homer and SHANK in mice. Such *in vivo* studies may help scientists determine whether abnormalities in the scaffolding complex could trigger behaviors of autism.

"Putting these findings in animal models is going to help us clarify the function, particularly for these two proteins, Homer and SHANK," says **Carlos Pardo-Villamizar**, an associate professor of neurology and pathology at the Johns Hopkins Hospital in Baltimore, who is not involved in the study. "It's critical to know if defects in the interactions of these two proteins occur in autism."

New techniques in animal models are making it easier to study other proteins in the PSD that are impaired in autism and other disorders. Researchers typically isolate these proteins using a technique that separates the molecules based on their affinity to specific antibodies. In many cases, antibodies also pull out unwanted protein contaminants, molecules that are tightly attached to the targeted molecule.

In contrast, a study published online 19 May in *Molecular Systems Biology* describes mutant mice carrying a gene that allows researchers use specific antibodies to label and isolate PSD-95, one of the proteins in the PSD⁵. Scientists can also use the technique to target other proteins within the PSD.

"We can isolate protein complexes with far fewer contaminants and without denaturing the proteins, so they retain biological activity," says lead investigator **Seth Grant** of the Wellcome Trust Sanger Institute in Cambridge, UK. His study was the first to use the new technique in mammals.

Grant's group is now working to breed the genetically tagged mouse with undisclosed mouse models of autism and schizophrenia. The offspring would have both the disease variant and a tagged PSD complex, allowing researchers to see directly how synapses are altered in the disorder.

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