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Researchers identify protein that initiates the formation of stable, long-term memories

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Prions can be notoriously destructive, spurring proteins to misfold and interfere with cellular function as they spread without control. New research, publishingin the open access journal *PLOS Biology* on February 11 2014, from scientists at the Stowers Institute for Medical Research reveals that certain prion-like proteins, however, can be precisely controlled so that they are generated only in a specific time and place. These prion-like proteins are not involved in disease processes; rather, they are essential for creating and maintaining long-term memories.

"This protein is not toxic; it's important for memory to persist," says Stowers researcher Kausik Si, who led the study. To ensure that long-lasting memories are created only in the appropriate neural circuits, Si explains, the protein must be tightly regulated so that it adopts its prion-like form only in response to specific stimuli. He and his colleagues report on the biochemical changes that make that precision possible.

Si's lab is focused on finding the molecular alterations that encode a memory in specific neurons as it endures for the days, months, or years—even as the cells' proteins are degraded and renewed. Increasingly, their research is pointing toward prion-like proteins as critical regulators of long-term memory.

In 2012, Si's group demonstrated that prion formation in nerve cells is essential for the persistence of long-term memory in fruit flies. Prions are a fitting candidate for this job because their conversion is self-sustaining: once a prion-forming protein has shifted into its prion shape,

additional proteins continue to convert without any additional stimulus.

Si's team found that in fruit flies, the prion-forming protein Orb2 is necessary for memories to persist.

Flies that produce a mutated version of Orb2 that is unable to form prions learn new behaviors, but their memories are short-lived. "Beyond a day, the memories become unstable. By three days, the memory has completely disappeared," Si explains.

In the new study, Si wanted to find out how this process could be controlled so that memories form at the right time. "We know that all experiences do not form long-term memory—somehow the nervous system has a way to discriminate. So if prion-formation is the biochemical basis of memory, it must be regulated." Si says. "But prion formation is random for all the cases we know of so far."

Si and his colleagues knew that Orb2 existed in two forms—Orb2A and Orb2B. Orb2B is widespread throughout the fruit fly's nervous system, but Orb2A appears only in a few neurons, at extremely low concentrations. What's more, once it is produced, Orb2A quickly falls apart; the protein has a half-life of only about an hour.

When Orb2A binds to the more abundant form, it triggers conversion to the prion state, acting as a seed for the conversion. Once conversion begins, it is a self-sustaining process; additional Orb2 continues to convert to the prion state, with or without Orb2A. By altering the abundance of the Orb2A seed, Si says, cells might regulate where, when, and how the conversion process is engaged. But how do nerve cells control the abundance of the Orb2A seed?

To look for leads, the scientists searched for proteins that physically interact with Orb2A. Because Orb2A is so scarce, finding it and

identifying its molecular accomplices took perseverance and refinement of the standard protein-cataloging techniques. Post-doctoral researcher Erica White-Grindley led that effort, and after several years, turned up more than 60 suspected partners for Orb2A.

One of these proteins, TOB, doubled the half-life of Orb2A, thereby temporarily increasing its abundance. The scientists were skeptical that this boost in stability would be enough to reliably stabilize long-term memories, but further biochemical analyses led them to a more satisfying explanation.

Their experiments revealed that when TOB associates with Orb2A – which is known to occur in response to an incoming nerve signal—this triggers the addition of chemical tags known as phosphates to both of the proteins, altering both proteins' stability. Once phosphorylated, the TOB-Orb2A complex falls apart and Orb2A becomes much more stable, with a new half-life of 24 hours. This increases the prevalence of the prion-like state.

This explained how Orb2's conversion could be specifically triggered following nerve cell stimulation. The next step was to determine how the process could be localized to the right neuronal connections. They found that the enzyme that places the phosphate tag on Orb2A is Lim kinase, a neuron-specific kinase that others had shown is activated at the synapse—the connection between neurons—when cells receive an impulse. Taken together, Si says, these experiments show how Orb2's conversion to the prion state can be confined in both time and space.

The findings raise a host of new questions for Si, who now wants to understand what happens when Orb2 enters its prion-like state, as well as where in the brain the process occurs. While unraveling these mechanisms will likely be more accessible in the fruit fly than in more complex organisms, Si points out that proteins related to Orb2 and TOB

have also been found in the brains of mice and humans. He's already shown that in the sea snail *Aplysia*, conversion to a prion-like state facilitates long-term change in synaptic strength. "This basic mechanism appears to be conserved across species," he notes.

More information: White-Grindley E, Li L, Khan RM, Ren F, Saraf A, et al. (2014) Contribution of Orb2A Stability in Regulated Amyloid-Like Oligomerization of Drosophila Orb2. PLoS Biol 12(2): e1001786. DOI: 10.1371/journal.pbio.1001786