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Identifying a Component of the Nerve Cell Scaffold

Researchers have discovered an important molecular component of the scaffold that supports and controls the release of chemicals involved in transmitting signals between nerve cells. The discovery offers a new pathway to understanding how the tiny sacs that contain neurotransmitters are managed in the nerve cell.

In their studies in mice, the scientists determined that the scaffold protein, called RIM, helps control changes in nerve signaling that enable the establishment of preferred neural pathways in learning and memory.

"But the discovery that RIM1 is one central component, if not *the* central component of the scaffold is a very important step."

— **Thomas C. Südhof**

The researchers published their findings in two articles in the January 17, 2002, issue of the journal *Nature*. Howard Hughes Medical Institute investigator Thomas C. Südhof at the University of Texas Southwestern Medical Center and Robert C. Malenka at Stanford University School of Medicine led the research teams.

According to Südhof, the researchers' aim was to understand the structure and function of a neuronal region called the "active zone" from which synaptic vesicles send neurotransmitter signals across the synapse, the junction between neurons.

Within the active zone, the signals from the transmitting nerve cell cause the synaptic vesicle to fuse with the cell membrane and release its cargo of neurotransmitter in a process called exocytosis. Researchers had previously identified some of the protein components of the scaffolding, but it was still largely a mystery how the proteins worked together.

"Work by our laboratory and others had shown that a protein called Munc-13 is an essential component of the active zone that is required for priming synaptic vesicles to undergo exocytosis, although it's unclear why," he said. "Also, it was known that Munc-13 binds to RIM which in turn binds to

another protein called Rab3a, which is also an important regulator of exocytosis.”

To understand more about the function of RIM1, the researchers produced knockout mice that lacked the most abundant form of RIM, called RIM1 α , and tested the effect the loss of that protein on transmission of nerve signals. The knockout mice survived, but the scientists observed that the animals failed to care for their offspring.

“The most complicated behavior that caged mice perform is to bring up their offspring,” Südhof said. “So, although we have not proved it, we suspect that abnormalities in this nurturing behavior indicate that these knockout mice have cognitive impairments due to dysregulated synaptic transmission.”

Biochemical analyses of synaptic proteins revealed that there was a considerable decrease in Munc-13 expression, yet the synapses appeared biochemically and structurally normal. “This means that the RIM protein, whatever it does, is not essential to make a synapse and is not needed to make the synapse look normal,” said Südhof.

In order to understand the role of RIM proteins in the active zone, the researchers searched for other active zone proteins that interact with RIM1 α . Their search identified several proteins that interact with RIM1 α , including the protein α -liprin, an important adapter that binds to enzymes in the active zone.

When the researchers studied the electrophysiological functioning of synapses in the knockout mice, they found that loss of RIM1 α caused abnormalities in the plasticity of the synapses – that is, their ability to regulate the strength of their responses, as occurs in learning and memory. These abnormalities included altered short-term plasticity – reflected in an impaired ability to change synaptic strength when subjected to paired stimulatory pulses. The researchers also observed that knocking out RIM1 α abolished a form of plasticity called long-term potentiation (LTP) that is a key mechanism underlying learning and memory. Specifically, the knockout mice lacked one particular form of LTP, called mossy-fiber-LTP, which depends on long-term increase in neurotransmitter signaling in the presynaptic to postsynaptic region.

Taken together, the findings reveal that RIM1 α plays an important role in the active zone, Südhof said. “I believe that this is the first time that we have developed a model that unites biochemical and physiological results obtained in mutant animals indicating that one particular protein, RIM1 α , plays a central role in pulling together different active zone components into a functional unit and integrating intracellular signals that regulate neurotransmitter release,” he said. However, he emphasized, the findings represent only a starting point for further research into the details of the active zone structure and function.

“What remains completely unclear, for example, is how does mossy-fiber-LTP work?” he asked. “What is the physical mechanism that

increases neurotransmitter release in this form of LTP? We don't know the answer to that from this paper, but with further research it will hopefully at some point be clarified. But the discovery that RIM1 is one central component, if not *the* central component of the scaffold is a very important step in making progress on this question as well as many other questions regarding the regulation of neurotransmitter release," said Südhof.