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RESEARCH NEWS

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Visualizing Protein Synthesis in Living Neurons

Over the last two decades, neuroscientists have been building the argument, buttressed by data from many experiments, that dendrites -- the fine fibers that extend from neurons -- can synthesize proteins. If dendrites can synthesize proteins, they may also have the capacity to modulate the strength of connections between neurons and ultimately influence neural activities, including learning and memory.

In a research article published in the May 2001 issue of the journal *Neuron,* Howard Hughes Medical Institute investigator Erin M. Schuman and colleagues at the California Institute of Technology describe a new technique that has allowed them to show that protein synthesis occurs in intact dendrites. The technique relies on a reporter molecule constructed by Schuman and colleagues, Girish Aakalu, Bryan Smith, Nhien Nguyen and Changan Jiang. When the molecule is introduced into neurons, it emits a telltale glow if protein synthesis is occurring.

The images produced by Schuman's team demonstrate that protein synthesis takes place locally in the dendrites. "There was early evidence that protein-synthesis machinery was present in dendrites," said Schuman. "Those findings were intriguing because they implied that dendrites had the capacity to make their own proteins."

The idea that dendrites should be able to synthesize proteins made sense to Schuman and others because it was more economical and efficient. "It's like the difference between centralized and distributed freight shipping," she said. "With central shipping, you need a huge number of trucks that drive all over town, moving freight from a central factory. But with distributed shipping, you have multiple distribution centers that serve local populations, with far less transport involved."

Previous studies had indicated that fragments of dendrites had the capacity to synthesize proteins. Schuman and her colleagues believed that visualizing local protein synthesis in living neurons would provide a more compelling picture than was currently available.

The scientists began their efforts to create a protein synthesis reporter by flanking the gene for a green fluorescent protein with two segments of a gene for an enzyme called calcium/calmodulin-dependent kinase II-α (CAMKII-α). By including CAMKII-α, the researchers ensured that they could target their protein synthesis reporter to dendrites.

In a series of experiments, the scientists inserted the protein synthesis reporter into rat neurons in culture, and then triggered protein synthesis using a growth factor called BDNF. By imaging the neurons over time, they showed that the green fluorescent protein was expressed in the dendrites following BDNF treatment -- unequivocal proof that protein synthesis was taking place. Going a step further, the researchers showed that they could cause the fluorescence to disappear by treating the neurons with a drug that blocked protein synthesis.

Schuman and her colleagues also addressed whether proteins synthesized in the main cell body, called the soma, could have diffused to the dendrites. "The soma is a potentially greater source of proteins than the dendritic spines, so we had to show convincingly that there was no way that the signal we were observing in the dendrites was coming from the soma." The researchers isolated the dendrites by snipping them from the neurons. The isolated dendrites also exhibited protein synthesis.

Schuman's group then created a version of the reporter that anchored the fluorescent protein to the cell membrane. When they used laser light to "photobleach" the cell bodies to eliminate any fluorescent protein, they still observed a prominent fluorescent signal in the processes. This, too, indicated that protein synthesis was occurring in the dendrites.

Intriguingly, said Schuman, hot spots of protein synthesis were observed within the dendrites. By tracking the location of the fluorescent signal over time, the researchers could see that these hotspots waxed and waned consistently in the same place in dendrites. "The main attraction of local protein synthesis is that it could endow synapses with the capacity to make synapse-specific changes, which is a key property of information-storing systems," said Schuman. "The observation of hot spots is consistent with this idea because it suggests that there are localized spots of protein synthesis that are near synapses that may provide new proteins to their associated synapses."

Schuman and her colleagues are now applying their protein synthesis reporter system to more complex brain slices and whole mice. "In the whole animals, we're exploring the role of protein synthesis in information processing and animal behavior," said Schuman. "For example, in the hippocampus, the brain structure we study, most neighboring neurons don't seem to have functional similarities if you're recording signals from their cell bodies. Neighboring neurons appear to like different kinds of stimuli. But using this protein synthesis reporter, we might uncover similar functional domains in the dendrites of these different cells that we wouldn't see if we were just

recording from their cell bodies," she said.