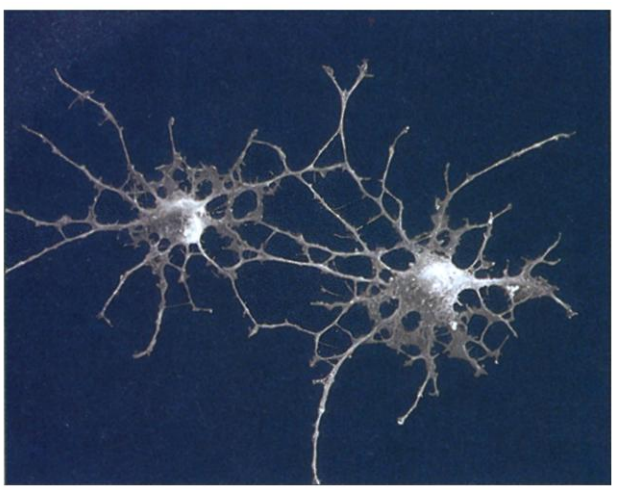
Synapses Call the Shots

Neuroscience research is shedding light on how neurons delegate their protein synthesis, shipping some messenger RNAs out to the dendrites, where they are translated to protein under the control of local synapses

"Dendrites are the brains of the neurons," neuroscientist Jim Eberwine of the University of Pennsylvania in Philadelphia likes to say. And with good reason. These highly branched structures do the neurons' computations, receiving and adding up signals coming in from other neurons through contacts called synapses. But dendrites do far more than just tally those signals; they also respond to them by strengthening or weakening their synapses. For example, repeated signals to a particular synapse may strengthen that synapse, making it respond more strongly to future signals; other activity patterns can weaken the synapse's future responses. These changes are believed to be at the very root of learning and memory, enabling the brain to learn from and adapt to the information it receives and to store the memories for future use.

These synapse changes require a supply of new proteins, and those proteins must be directed to specific synapses, because synapses undergo changes independently of their neighbors. That presents neurons with a distribution challenge, because the dendrites form a finely branched maze whose synapses may be hundreds of micrometers from the neuron's cell body. Now, after decades of piecing together hints, neuroscientists are building a detailed picture of how this made-to-order protein synthesis occurs. The emerging picture is one in which synapses have "some kind of autonomy," says neuroscientist Kelsey Martin of the University of California, Los Angeles (UCLA). "You are regulating gene expression very locally within a single cell." Indeed, it appears that finely controlled, decentralized protein production may contribute to learning and memory in a way that neuroscientists could scarcely have imagined 20 years ago.

That's because the new findings provide a fresh view of the balance of power within cells. Biologists have traditionally viewed the nucleus and its surroundings as the command center of the cell, with the nucleus supplying messenger RNAs (mRNAs) to the ribosomes, tiny protein factories that wait nearby to translate the mRNAs into proteins. Although that general scheme operates in all cells, it appears that neurons have adopted an additional strategy for supplying proteins to their distant dendrites: mRNAs are marked for delivery to the dendrites, where ribosomes function as protein-production outposts; both transport and production are under the control of nearby synapses.



Routing riddle. Highly branched dendrites like these provide a navigational challenge for messages and materials traveling between synapse and nucleus.

First inklings

This revolution in thinking hasn't come overnight. For nearly 2 decades researchers have had evidence that proteins may be made in the dendrites. But recent experiments are fleshing out the fine points, showing how neurons mark mRNAs for transport to the dendrites from the nucleus, and how the activity level of synapses in the dendrites regulates that transport and the mRNAs' eventual translation.

The excitement over protein synthesis in the dendrites began in 1982, when neuroscientist Oswald Steward, then at the University of Virginia in Charlottesville, noticed that electron micrographs (EMs) of dendrites showed ribosomes apparently linked to mRNAs, which suggested that the dendrites were making protein. What's more, says Steward, now at the University of California, Irvine, "the localization of the ribosomes was selective." They weren't just anywhere in the branching dendritic tree, they were in the dendritic spines, the fine, twiglike projections that form synapses. The ribosomes seemed perfectly positioned to supply the proteins synapses need to make the long-term changes that are key to learning and memory. "He showed definitely that the protein-synthetic machinery was there," says Brown University neuroscientist Justin Fallon. And that provided "a gorgeous mechanism" for regulating the production of synapse-specific proteins, adds neurobiologist Mark Mayford of the Scripps Research Institute in La Jolla, California.

The EMs certainly suggested that proteins were being made in the dendrites, and other researchers verified by immunostaining that all the necessary components of the protein-producing machinery were there. But it wasn't until 1996 that researchers got direct evidence for that protein synthesis. Eberwine and postdoc Peter Crino did this by severing the dendrites of cultured neurons and sucking the cell bodies out of the culture dishes, leaving the disembodied dendrites behind. They then injected mRNAs into the dendrites and showed that those mRNAs were translated into protein.

The missing link

But many questions remained. Perhaps most important, researchers had not shown a link between dendritic protein synthesis and the synapse changes associated with learning. Among the first groups to forge that link was Erin Schuman's at the California Institute of Technology (Caltech) in Pasadena. The team had shown that a protein called brain-derived neurotrophic factor (BDNF) strengthens synapses in the hippocampus, a brain area associated with some kinds of learning. The strengthening requires fresh proteins, and to learn whether those proteins are made in the dendrites or the cell body, the team took advantage of a feature of hippocampal anatomy: The cell bodies of certain neurons are in one layer of the hippocampus, but their dendrites are in another layer, some distance away. In experiments reported in 1996 the researchers simply sliced the layers apart in cultured hippocampal tissues, disconnecting the dendrites from their cell bodies. When they added BDNF to the cultures, it still strengthened the synapses, which meant, Schuman says, that the disconnected dendrites "had to be the site of protein synthesis."

Experiments like Schuman's did not reveal the identity of the proteins made in the dendrites, but other researchers were working on that question. One protein they found is calcium-calmodulin–dependent kinase II (CaMKII), an enzyme that adds phosphate groups to other proteins. CaMKII is a hot research topic because it plays a key role in the synapse strengthening linked to learning and memory; when it turned out to be translated in the dendrites, it became a model protein for studying that process as well. Last year, Mary

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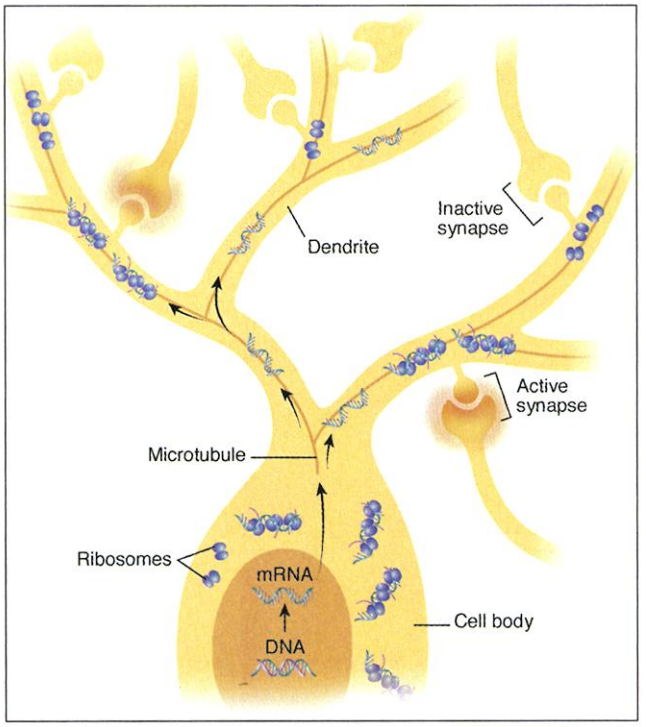
Kennedy, postdoc Yannan Ouyang, and their colleagues at Caltech showed that CaMKII is made in the dendrites of neurons whose synapses have been stimulated in a way that triggers long-term potentiation (LTP), a form of synapse strengthening that appears to be involved in certain kinds of learning. Using antibodies to CaMKII, they detected a 30% increase in the amount of the protein in the dendrites after triggering LTP. They were able to block the increase with protein synthesis inhibitors, suggesting the increase was indeed due to new protein synthesis.

Although CaMKII synthesis had already been shown in the dendrites, Kennedy's experiment provided important evidence that synaptic activity could boost that synthesis. What's more, the translation that is boosted had to be local, Kennedy says, because the new CaMKII began to appear in the dendrites within 5 minutes of the stimulation—much too fast for it to have arrived from the cell body. The idea that synapse activity triggers local enzyme synthesis was reinforced in March, when A. J. Scheetz, reporting in *Nature Neuroscience* on work he did as a postdoc in Martha Constantine-Paton's lab at Yale University, showed that the new CaMKII protein was made when he activated synapses in synaptoneurosomes, which are preparations of intact synapses that have been broken off from their cell bodies.

The Scheetz team's work also suggests how synapse stimulation turns up translation of the CaMKII mRNA, an important issue because this mRNA is always present in high concentrations in the dendrites. Scheetz and Constantine-Paton found that stimulation actually turns down overall dendritic translation by inhibiting a part of the translation machinery called an elongation factor. That makes elongation the rate-limiting step in protein translation and allows proteins whose mRNAs are abundant in the dendrites, like those of CaMKII, to tie up more of the ribosomes and make proportionately more protein. "It is a way of having a pool of transcripts available but not utilized" until they are needed, Scheetz says. Some viruses use a similar strategy, flooding host cells with their own mRNA while repressing production of host proteins.



Production outposts. mRNAs are transported on microtubules to the dendrites, where ribosomes translate them into protein. The activity level of synapses controls the process.

A second mechanism revealed

CaMKII had more lessons to divulge, including a second means of translation control operating in the dendrites—a means that neurons share with developing oocytes. Brown's Fallon, working with Joel Richter of the University of Massachusetts Medical School in Worcester, found a code in the tail end of CaMKII mRNA—the so-called 3′ untranslated region—that appears to trigger the mRNA's translation.

Richter's lab studies translation in developing oocytes, in which some mRNAs have a sequence in their 3′ untranslated region called the cytoplasmic polyadenylation element (CPE). When a protein called CPEB binds to the CPE, that triggers the addition of a string of adenine nucleotides to the RNA's tail (polyadenylation), which in turn activates the mRNAs for translation. Knowing that translation occurs in dendrites, Fallon encouraged Richter to look for CPEB there. The team found it.

Then Fallon and his postdoc Dave Wells joined forces with Richter and his postdoc Lin Wu and found that the CaMKII 3′ untranslated region contains a CPE. What's more, when they strengthened synapses in the brains of young rats, CaMKII mRNA became polyadenylated and newly made CaMKII appeared at the synapses. To see if the increased synthesis was due to polyadenylation, they mutated the CPE. That mutation abolished the 3′ untranslated region's ability to increase protein translation in cultured neurons, confirming their hypothesis.

Fallon says the elongation factor and polyadenylation schemes for boosting translation are "not at all incompatible." The phenomenon that Scheetz and his colleagues observe is "all over within 30 minutes," he says, and that is when his team just begins to see polyadenylation. Modification of the elongation factor may provide the first boost to CaMKII translation, he

Translational Roots for Mental Retardation?

A flurry of findings points to protein translation in the dendrites of neurons as a key feature leading to the changes at synapses that are vital to learning (see main text). And one recent discovery suggests that when this translation goes awry, it can lead to mental retardation. In 1997, William Greenough and Ivan Jeanne Weiler of the University of Illinois, Urbana-Champaign, working with Jim Eberwine of the University of Pennsylvania in Philadelphia, surveyed the messenger RNAs (mRNAs) found in synaptoneurosomes, preparations of intact synapses broken off from their cell bodies. One mRNA turned out to encode the fragile X mental retardation protein (FMRP), whose gene is mutated in fragile X syndrome, the most common inherited form of mental retardation. What's more, the researchers found that this mRNA became linked to ribosomes—an indication that it was being translated into protein—just minutes after they stimulated the synapses. New FMRP protein also appeared after stimulation, confirming that the protein's production is triggered by synaptic activity.

The work may shed some light on how FMRP mutations cause mental retardation. FMRP is an RNA-binding protein, and recent findings in Greenough's lab suggest that FMRP is needed for protein synthesis near synapses in response to stimulation. When his team stimulated synaptoneurosomes from mice that lack the FMRP gene, the stimulation didn't boost translation as it does in synaptoneurosomes from normal mice. "That suggests that the protein plays a positive role in regulation of translation," Greenough says. But "the real story from the clinical point of view," he adds, "is going to be [finding out which] proteins are regulated." **–M.B.**

ILLUSTRATION: C. CAIN

suggests, and mRNA polyadenylation may take over later.

Mail order

Regulating translation is just one way that active synapses boost their protein supplies. It appears to be a key role for proteins like CaMKII, whose mRNA is found at high levels in the dendrites whether or not synapses are stimulated. But researchers studying another mRNA uncovered a whole different level of regulation, in which active synapses order up more mRNA to be shipped out to them from the nucleus. This was dramatically demonstrated for activity-regulated cytoskeletal-associated protein (Arc). In 1995, Greg Lyford, Paul Worley, and their colleagues at Johns Hopkins University in Baltimore, Maryland, found that Arc mRNA is quickly made in the nuclei of neurons that have undergone LTP—hence the designation "activity-regulated"—and shows up in the dendrites. Steward joined Worley to see if the mRNA goes specifically to active synapses.

The researchers used a trick to trigger LTP in one of two layers of the hippocampus of rats, depending on where they placed their electrode. After triggering LTP in one layer, they stained slices of the rat's brains for newly synthesized Arc mRNA and found that it concentrated specifically in the layer that had undergone LTP. If they triggered LTP in the other layer, the Arc mRNA went there. At both sites, it was translated into Arc protein. "The really cool thing was that it went to the activated synapses," says Brown's Fallon. Neuroscientist Kenneth Kosik of Harvard Medical School in Boston agrees: "For RNA to navigate in that [dendritic] tree, say, to take the first left- and then two right-hand turns and get to the right synapse—it is just an extraordinary regulatory process that is going on there."

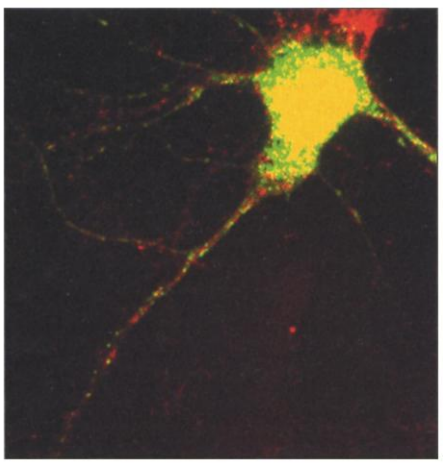
Postal codes

While the mechanism that directs proteins precisely to activated synapses remains a mystery, it is clear that any mRNA that travels to the synapses must carry a sequence that serves as a general "postal code" to direct it out to the dendrites. Several teams have searched for that code. In 1996, Mayford of Scripps, who was then a postdoc in Eric Kandel's lab at Columbia University, decided that "the likeliest place for a signal to be was the 3′ untranslated region," where other regulatory codes have been found.

To test that hunch, he spliced the DNA encoding the 3′ untranslated region from the CaMKII mRNA to DNA encoding a bacterial enzyme that turns tissue blue. When he put the hybrid gene into mice, he found blue color in the dendrites of the animals' brains. That meant the 3′ untranslated region was sufficient to direct the mRNA to the dendrites. Conversely, as Stephan Miller, a postdoc in Mayford's lab, will report at the upcoming Society for Neuroscience meeting in New Orleans, swapping the CaMKII mRNA's usual 3′ untranslated region for another RNA sequence effectively locks the mRNA in the cell body and keeps it from finding its way to the dendrites.

This appears to be a widely used mechanism for directing mRNAs to the dendrites. For instance, last year Stefan Kindler's team at the University of Hamburg in Germany reported that part of the 3′ untranslated region of the mRNA for microtubule-associated protein-2 directs that mRNA to the dendrites.

That 3′ untranslated region code apparently works by linking mRNAs to microtubules,



Shuttle. Fluorescently labeled mRNAs (green) make their way into the dendrites. Red labeling shows the synapses.

fibers that form a sort of monorail system for transporting materials around cells. Harvard's Kosik and postdoc Martha Rook recently devised a scheme for watching the movements of specific mRNAs in living neurons. They hooked the 3′ untranslated region from the CaMKII mRNA to an RNA sequence that binds a fluorescent protein. As they reported in the September *Journal of Neuroscience*, they saw the fluorescently labeled RNAs move gradually into the dendrites of cultured neurons with an oscillating motion. When they activated the synapses, the outward movement of the mRNAs was "more directed," says Kosik. "They seemed to go over a longer distance without movement back." (See movies of the RNA movements at neuro-oas.mgh.harvard.edu/alzheimers/Rooketal/rook_et_al.html)

Wondering which proteins might escort the dendrite-bound mRNAs on their monorail journey, researchers turned to an RNA-binding protein called Staufen. Staufen was discovered shuttling mRNAs in developing fruit fly embryos, but researchers later found a mammalian form of Staufen that appears in brain neurons. Early last year, Michael Kiebler, Carlos Dotti, and their colleagues at the European Molecular Biology Laboratory in Heidelberg, Germany, reported that Staufen is present both in RNA-containing particles in the cell body and in dendrites of hippocampal neurons from rat brains. Evidence that Staufen might help transport mRNAs to the dendrites of rat neurons came last fall when Kiebler's team, now at the Max Planck Institute in Tübingen, Germany, showed that it travels on microtubules into the dendrites. And Caltech's Schuman reported at last year's Society for Neuroscience meeting that neurons carrying a defective form of Staufen had less mRNA in their dendrites.

Another unresolved issue concerns how mRNAs know which synapses to go to in the vast mazelike network of dendrites. An mRNA heading for a specific synapse must make many choices at forks in its path, and that clearly requires more than just a dendrite-specific shipping label. When the synapse sends its request to the nucleus, says Schuman, "it is possible that some kind of information about the identity of the synapse that sends the signals could also be transported," leaving a sort of breadcrumb trail that the mRNA can follow back. But no one yet knows what that information might be.

Once the mRNA finds the right neighborhood, several teams have evidence that activated synapses wear tags that direct mRNAs to their doors. UCLA's Martin, working with Andrea Casadio as postdocs in Kandel's lab, got a clue as to the nature of the tags when they showed that synapses from the sea slug *Aplysia* can mark themselves even when protein synthesis is blocked. That, says Martin, means the tags must not be newly made proteins. Instead, the researchers suggest that the tags may be placed by an existing enzyme, perhaps a kinase that tacks phosphate groups onto the activated synapse.

One big unanswered question is how activated synapses place their orders with the nucleus for more mRNAs. Martin, Casadio, and Kandel found that when they blocked protein synthesis at the synapses, synapse activation no longer triggered long-term synaptic changes. Those changes require gene activation in the nucleus, says Martin, a result that "tells us that local protein synthesis is involved in the feedback" that triggers that gene activation. What's more, Kandel's and Eberwine's teams have found that the dendrites make a variety of proteins known to regulate gene transcription in the nucleus; it is possible that these proteins may be the messengers.

As researchers press on, they are filling in a picture of a complex loop of communication and supply, with synapses acting as local managers wielding more power than anyone might have imagined, to get what they need for carrying out the critical task of storing memories.

–MARCIA BARINAGA

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