RNA and the synapse

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One reason we learn and remember is because synapses, the places where axons and dendrites meet, undergo experience-dependent changes whose long-lasting nature may underlie the formation and persistence of memory. Indeed, neurons use these morphological and biochemical alterations to distinguish experienced (i.e., stimulated) from naïve (unstimulated) synapses, thereby forming a cellular basis of learning and memory. How synapses change—i.e., plasticity—is an obviously complicated affair but a breakthrough study by Erin Schuman's lab in 1996 set the stage for what is now the burgeoning field of local translation in neurons. These investigators treated slices of the rat hippocampus (a part of the brain required for memory processing) with the neurotrophin BDNF and measured long term potentiation (LTP), one form of synaptic plasticity. BDNF induced LTP, but not when the slices were also treated with anisomycin, an inhibitor of protein synthesis. Physical separation of the nucleus-containing cell body from the axon and dendrite-containing neuropil layer had little effect on LTP evocation by application of BDNF to the neuropil again. However, treatment of the neuropil with BDNF and anisomycin failed to induce LTP, indicating that local protein synthesis, presumably in dendrites because those structures contain the preponderance of polyribosomes, is required for plasticity and, by extension, learning and memory. To my mind, this paper is seminal because it firmly demonstrated the importance of translational control in plasticity and because it launched many investigations into how local translation mediates plasticity, how dendritic translation is regulated, how local translation might be involved in neurologic disease.

Because I find these topics particularly exciting, I will discuss a few findings that piqued my interest or were fundamental discoveries that opened new doors into how RNA influences the workings of the nervous system. But first, a few qualifiers: because of space limitations, I will focus primarily on synapse function and learning and memory; I will not discuss such relevant topics as axon guidance, which is also regulated by local protein synthesis, or motor neuron dysfunction, which is controlled by RNA binding proteins. Some terrific reviews of these subjects have been published

by Christine Holt, Maury Swanson, Don Cleveland, Tom Cooper, and Gideon Dreyfuss and are easily found in PubMed.

LTP has two phases: an early one that involves posttranslational modifications of extant proteins and a late one that is protein synthesis-dependent. LTP strengthens synaptic connections, which facilitates communication among neurons and thus presumably promotes memory consolidation. The other side of the coin is a plasticity that weakens synaptic connections; this is long term depression (LTD). Using an experimental set-up similar to the one described earlier, Mark Bear and colleagues found that LTD also required local protein synthesis in the neuropil region of the hippocampus. How can LTP and opposing LTD both require translation in dendrites? LTP can be induced multiple ways but the stimulus usually promotes calcium entry into the postsynaptic compartment, which activates calcium-calmodulin kinase II (CaMKII) signaling cascades. LTD can also be stimulated several ways, one of which is by the treatment with an agonist of metabotropic glutamate G-protein coupled receptors that activates signaling events through inositol-3-phosphate (IP3). One would therefore surmise that stimulus-dependent mRNA-specific translation in dendrites mediates these different forms of plasticity, however, there is significant kinase crosstalk between the downstream signaling events and it has yet to be established that there are distinct LTP and LTD mRNAs.

What are the mRNAs that could mediate synaptic plasticity? Two studies took different approaches to identify dendritic mRNAs. Kelsey Martin's lab cultured primary neurons on filters with sufficient pore size through which dendrites and axons could grow, thereby effectively separating the neuronal processes from the cell body. Microarray analysis of the RNA from the collected axons and dendrites revealed over 100 distinct sequences in these processes many of which encode translation factors and RNA binding proteins. In contrast, the Schuman lab found over 2500 mRNAs localized to dendrites and axons following dissection of the rat neuropil and deep sequencing of the extracted RNA. Although they also detected mRNAs encoding translation factors and RNA binding proteins, most of the sequences

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they found encode synaptic components and signaling molecules often found at the synapse. The discrepancy between the two studies could be the result of in vitro versus in vivo analysis, the degree of enrichment of neurite processes, and/or the depth of the RNA sequence.

One might ask whether dendrites contain dormant mRNAs that are translated only when stimulated to do so. This phenomenon of regulated translation was recently demonstrated by Rob Singer and colleagues who used protease treatment of cultured neurons to remove proteins from β -actin mRNA combined with high resolution fluorescence in situ hybridization (FISH) to (in my opinion) unequivocally demonstrate mRNA unmasking in dendrites in response to chemical induction of LTP.

Translational stimulation of dormant mRNAs has long been studied in *Xenopus* development and the similarities between this process in oocytes and dendrites are striking. Both dendrites and oocytes contain masked mRNAs, which in response to external cues (synaptic activity in the case of dendrites and progesterone stimulation in the case of oocytes), undergo poly(A) elongation and translation. Our lab has shown that in both systems, the RNA binding protein CPEB regulates polyadenylation in a phosphorylation-dependent manner and does so in conjunction with at least two enzymes that mediate poly(A) length: the poly(A) polymerase Gld2 and the deadenylating enzyme PARN. Many components of the oocyte CPEB/cytoplasmic polyadenylation complex are also found at synapses and several of them regulate LTP. Interestingly, CPEB regulates both LTP, LTD, and learning and memory, perhaps suggesting that it is near the top of a regulatory hierarchy affecting the expression of different RNAs that result in LTP or LTD.

Another form of translational regulation that is gaining traction in many systems including neurons is at the level of polypeptide elongation (ribosome transit). Over a dozen years ago, data from Ken Kosik's lab suggested that dormant mRNAs in the brain were associated with ribosomes and that KCl-induced membrane depolarization seemed to mobilize potentially stalled ribosomes to continue to elongate polypeptides. Wayne Sossin's group performed a neuron in situ analysis using ribopuromycylation and "click-it" chemistry to label de novo synthesized polypeptides to show that LTP induction mobilized stalled ribosomes. Activation of stalled ribosomes has now been shown in a number of systems and this regulatory process seems poised to explode just as the regulation of initiation did two or three decades ago.

One particular neurologic disease that has been linked to ribosome stalling for many years is the Fragile X syndrome. Fragile X, which affects 1 in 4000–5000 children, lies on the autism spectrum and is characterized by a range of pathologies including mental impairment (IQs can be as low as 40), attention deficits, developmental delays, epileptic seizures, certain physical abnormalities, and others. Fragile X is caused by a triplet repeat expansion in the *FMR1* gene, which leads to DNA methylation and transcriptional inactivation. *FMR1* encodes FMRP, an RNA binding protein that inhibits translation. Many labs have shown that the preponderance of FMRP co-sediments with polysomes in sucrose gradients, suggesting that it stalls ribosomes. A groundbreaking study from Jennifer and Bob Darnell's lab has not only given strong credence to the "stalled ribosome hypothesis" of FMRP, but has yielded insight into how this could occur. The Darnell experiments showed that FMRP not only UV-crosslinks (i.e., CLIPs) to about 1000 mRNAs in the brain, but mostly does so to coding regions. There appears to be no specific *cis* element to which FMRP CLIPs (although the Tom Tuschl lab, using cultured cells in which FMRP was ectopically-expressed, does find preferential binding sites), but instead seems to spread throughout the open reading frame. One could therefore surmise that FMRP acts as a roadblock to impede ribosome transit under normal conditions but that when *FMR1* is silenced and FMRP is not produced, polypeptide elongation would proceed at an elevated rate. Our lab has demonstrated that is precisely what occurs. Thus, Fragile X appears to be a disease of excessive ribosome movement. Indeed, this link between the Fragile X disease state and ribosome movement was strengthened by our observations that ablation of both FMRP and CPEB not only rescues Fragile X phenotypes in mice, but restores normal rates of ribosome transit. Finally, I want to note a potentially important study from Simpson Joseph and colleagues who used cryoelectron microscopy to show that *Drosophila* FMRP directly binds the ribosome via rpL5 in the 80S monosome. This result also indicates that FMRP impedes ribosome movement and it will be extremely interesting to see how the Darnell, Richter, and Joseph data can be brought together.

My friend Justin Fallon once said to me (and I paraphrase) "sooner or later you will be a neurobiologist." I'm not sure I'll ever be a neurobiologist, but judging from the explosion of molecular biology labs working in the nervous system it appears that many have heard a clarion call that RNA biology has much to contribute to this exciting field.

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