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mRNA translation: regulating an out of soma experience

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Abstract

The regulation of protein synthesis in discrete cellular subdomains, or local protein synthesis, has important roles in development as well as brain function. This review will discuss some recent findings that shed new light on mRNA translation regulation and how these layers of regulation may work together to elicit tissue specific and spatially restricted gene expression.

Introduction

In neurons, the regulation of mRNA translation outside the cell soma has garnered a great deal of attention as a way to modulate function of micro-domains such as the growing tips of axons and dendritic spines. The axonal growth cone and dendritic spine share a number of characteristics; they are both actin-rich structures, they respond to external cues, and they both modify their morphology in response to external cues, and in many cases these structures are far removed from the protein synthetic machinery of the soma.

The first clues that new proteins could be synthesized in axonal growth cones and dendrites came in the 1970's and 80's (respectively) with the identification of ribosomes in these distal processes [1,2]. Many reports in the subsequent decades described significant progress in the identification of transport and translational mechanisms regulating the synthesis of proteins in growth cones and dendrites. The current review will not attempt to summarize all this work, but rather focus on more recent findings that suggest novel mechanisms of regulation and the interplay between protein synthesis and degradation.

Localizing mRNA and ribosomes to the synapse

In neurons, a subset of mRNAs are thought to be transported into the periphery. For this cadre of mRNAs, once synthesized in the nucleus, they are identified for transport into the neuronal process by mRNA binding proteins that repress translation and incorporate the mRNA into ribonucleoprotein (RNP) particles for transport in granules [3]. Many mRNA-binding proteins have been identified that have a role in either transport, translation or both [4]. Recently, a cohort of mRNAs transported into dendrites were identified that retain intronic sequences containing ID elements, a form of SINE (short interspersed repetitive elements) retrotransposon [5]. mRNA isolated from dendritic regions of neurons in culture contained these ID elements, and when ID elements from several genes were cloned and expressed in neurons they were capable of targeting the exogenous RNA into dendrites. This exciting finding not only provides a novel mechanism for mRNA targeting into dendrites, it

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also raises the possibility that mRNA processing (splicing) may occur on-site at or around the synapse.

Functionally, synaptic mRNA translation plays a role in synaptic plasticity. Protein synthesis is required for many forms of long-term potentiation (LTP) and long-term depression (LTD) [6], and local synthesis was convincingly shown to play a key role in this process [7]. To determine how the PSD protein milieu is altered following LTP, Zhang et al. (2012) chemically induced LTP in cortical neurons in culture and used stable isotope labeling by amino acids in cell culture (SILAC) to detect changes in protein levels in the PSD. Interestingly, nearly 1/3 of the proteins translocated to the PSD were mRNA binding proteins [8]. This study was done in the presence of cycloheximide so does not include the contribution of newly synthesized proteins, but rather highlights the importance of recruiting mRNA to synapses following LTP-inducing stimuli. Indeed, several members of a large family of mRNA-binding proteins called heterogeneous nuclear ribonucleoproteins (hnRNPs) were detected in this assay, including hnRNPG and M [8]. Both have been implicated in mRNA splicing and support the idea that more intricate mRNA processing may be occurring in the dendrite than previously appreciated.

This recruitment of mRNA coincides nicely the recruitment of polyribosomes into spines following LTP-inducing stimuli in the hippocampus. Here, the Harris lab, using serial section electron microscopy (EM), reconstructed regions of dendrites in the hippocampus comparing high frequency (LTP-inducing) stimuli to hippocampal neurons receiving low frequency stimulation. Following high frequency stimulation, the number of spines that contained polyribosomes increased from 12% to 39% while the polyribosome profiles in the dendrite shaft decreased by a similar amount [9]. No change in the number of spines was detected; however, the polyribosome containing spines were larger. Suggesting that local protein synthesis was contributing to increased spine and PSD size [9,10].

While a tour de force, the Harris group studies have been limited to the identification of polysomes, leaving open the location/role and regulation of monosomes. In an intriguing examination of the localization and regulation of ribosomes in local translation, Tcherkezian et al. (2010) demonstrated a surprising interaction between protein synthetic machinery and DCC (deleted in colorectal cancer), a netrin receptor involved in axon guidance. Here, they show that the cytoplasmic tail of DCC interacts directly with the ribosomal protein L5. DCC also interacts with cap-dependent translational regulators like eIF4E and eIF3e and co-sediments with both 40s, 60s and 80s (monosome) fractions, but not polysomes [11]. Further, netrin stimulation dissociates the interaction, presumably freeing the translational components to form polysomes and thus actively translate mRNA [11]. Taken together, these data suggest that following plasticity-inducing activity, specific mRNA are recruited to the spine through their association with RNA binding proteins and polysomes are formed from the membrane associated components now freed from sequestration.

Ribosome regulate thyself

The sequestration/localization of the ribosome by DCC also hints at a novel regulatory component of protein synthesis only now being appreciated. The ribosome itself has for decades been thought of as the assembly line robot of protein synthesis, mindlessly pumping out product, but having constitutive rather than regulatory activity. While the ribosome is an elegant and complex RNP machine, the role of regulating protein synthesis has been deemed the prerogative of others – most notably mRNA binding proteins, initiation and elongation factors, and small non-coding RNA. However, recent findings in yeast [12], plants [13] and now in mice [14] have demonstrated that all ribosomes are not created equal.

In the budding yeast *S. cerevisiae* many (59 of 79) of the ribosomal proteins are encoded by two genes called paralogs (a remnant of an ancient genome duplication). The paralogous genes were thought to be functionally redundant as in many cases growth rate defects seen in deletion of one of the pairs can be rescued by overexpression of the other [15]. However, using a more sensitive assay, examining the localization of ASH1 mRNA, Komili et al. (2011) showed clear defects in ASH1 mRNA localization and bud site selection in gene knockouts that were not altered by overexpression of the duplicate gene. The aberrant ASH1 mRNA localization was detected in only a subset of ribosomal proteins and was not correlated with an alteration in ribosome assembly. Thus, it suggests that the paralogous proteins had distinct functions, even though their amino acid sequences were very similar or in some cases identical [12]. To further generalize this specific observation, the authors examine the transcriptome of yeast strains deficient in paralogous genes. They reveal a substantial variation in proteins enriched or repressed between paralogous ribosomal proteins. In some cases the categories of genes affected in the paralogous gene knockouts were completely independent [12]. This work suggests the existence of a “ribosome code”, i.e., the makeup of individual ribosomes could tune the ribosome to specifically or preferentially translate a subset of mRNAs.

This ribosome code (or ribosome filter as they termed it) was theorized by Mauro and Edelman in 2002 largely on the observation that some mRNAs contained complementary sequences with 18S or 28S rRNA [16]. They posited that small differences in ribosomal composition could lead to tissue-specific differences in gene expression [17]. In an elegant study, Kondrashov et al. (2011) provide evidence that loss of a ribosomal protein (RPL38) has tissue specific consequences directly related to the loss of expression of specific HOX genes. They demonstrate that deletion of RPL38 results in the developmental defects seen in the tail-short (*TS/+*) mutant mice. Interestingly, *TS/+* mice do not show global changes in protein expression, rather the expression of specific HOX homeoproteins are downregulated in select tissues while the mRNA levels encoding these proteins are unchanged. In fact, the majority of the developmental deficits in the *TS/+* mutant can be accounted for by the combinatorial loss of the affected HOX genes [14]. In addition, the expressed HOX mRNA could be efficiently translated in an *in vitro* system showing the deficit was not with the mRNA transcripts themselves [14]. This work again points to specialization of the ribosome that tunes translation toward specific subsets of mRNAs. Thus, the ribosome itself maintains the ability to regulate mRNA translation in a highly significant way. The mechanism(s) for how these regulations are manifest is still unclear but ultimately one would like to know how the ribosomal code works with RNA binding proteins and microRNA to regulate tissue specific and cell domain specific expression.

Yin and Yang

Both protein synthesis and degradation are required for many forms of synaptic plasticity and memory [18]. Functionally, degradation could be required for many aspects of plasticity. For example, in order to facilitate morphological changes, structure may have to be torn down prior to rebuilding [19], or proteins limiting synaptic strength (e.g., Arc) may first need to be degraded to facilitate receptor insertion [20]. However, two recent studies point to a direct connection between ubiquitin, the proteasome and protein synthesis. These studies are important because they illustrate that degradation and synthesis are not necessarily on parallel paths, but may have coordination such that each relies on the other for functional impact.

Much like the recruitment of RNA-binding proteins and polyribosomes to spines described above, the proteasome is also recruited following synaptic activity [21]. In a compelling recent report, Banerjee et al. (2009) demonstrate a mechanism whereby the synthesis of proteins is directly mediated by the degradation of MOV10, a homologue of the DExD-box

protein Armitage. MOV10 localized to synapses in hippocampal neurons is poly-ubiquitinated in response to NMDA receptor activation and degraded. They devised a “translational trap” to identify mRNAs that are recruited to the polysome fraction in the synaptodendritic compartment following the loss of MOV10 (via RNAi). When ranked by fold change, two of the top five mRNAs that were identified encoded proteins already shown to be regulated in dendrites and bound by miRNAs (Limk1 and α -CaMKII), suggesting a validation of the approach [22]. To further validate the approach, they examined Lypla1, a depalmitoylating enzyme not known to be regulated by activity. They showed that Lypla1 protein is increased in MOV10 knockdown and this regulation is mediated by Lypla1 mRNA interaction with miR-138. In addition, the activity-dependent synthesis of reporter constructs harboring either the Lypla1 or α -CaMKII 3'UTR (containing the miR regulatory domains) was reduced in the presence of the proteasome inhibitor lactacystin [22]. This work elegantly demonstrates a direct functional interplay between ubiquitin-mediated degradation and protein synthesis at synapses.

In a twist on ubiquitination of proteins in dendrites, the Kandel group has recently demonstrated a direct link between ubiquitination and protein synthesis. Here, they describe the function of neuralized1 (Neur1), an E3 ubiquitin ligase, in mouse hippocampus [23]. Instead of polyubiquitinating its target, Neur1 monoubiquitinates CPEB3 – an RNA-binding protein – leading to the activation of translation of CPEB3 bound mRNA. CPEB3 is a member of the cytoplasmic polyadenylation element binding protein family of proteins. The charter member being CPEB1, a protein that can both inhibit or activate mRNA translation depending upon its phosphorylation status [24]. CPEB3 does not have the consensus phosphorylation site seen in CPEB1, thus it was unclear how CPEB3 might regulate its target mRNAs [25]. Pavlopoulos et al. (2011) shows that Neur1 ubiquitination of CPEB3 leads to the polyadenylation and translation of two target mRNAs encoding subunits of the AMPA type glutamate receptor. CPEB3 is the homologue of the Aplysia CPEB which has been ascribed prion-like activity [26]. How (or if) ubiquitination regulates CPEB3 stability and/or aggregation is still unknown, but this work helps to define an important mechanism for protein synthesis in neurons.

Conclusion

In all, a model for protein metabolism at the synapse is emerging that suggests a complicated interplay between mRNA binding proteins, ribosomal regulation and protein degradation. How these mechanisms are coordinated spatially and what the key components are that trigger the polarity of the response (be it LTP or LTD) are still open and important questions. The emergence of a ribosome code could suggest that local expression of new proteins requires the translational availability of specific mRNAs and the coordinate activation of ribosomes tuned for the preferential translation of those mRNAs.

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