Presynapses go nuclear!

Dana O Kravchick¹ & Bryen A Jordan^{1,2}

Decades of research has shown that long-term changes in synaptic function ultimately require changes in gene expression. Recent work has focused on nuclear signaling by calcium and protein messengers initiated at postsynaptic sites. In this issue of *The EMBO Journal*, Ivanova and colleagues show that shuttling of CtBP-1 between presynaptic areas and nuclei regulates gene expression, which reminds us that presynaptic zones should not be ignored when considering synapse-to-nucleus signaling.

See also: **D Ivanova *et al*** (April 2015)

In 1967, Bernard Agranoff and Ramon Lim showed that inhibition of transcription using actinomycin D blocked the formation of memories in goldfish (Agranoff *et al*, 1967). This seminal work launched numerous studies to understand the role of gene expression in synaptic plasticity, learning and memory (Jordan & Kreutz, 2009; Kandel, 2012; Kaushik *et al*, 2014; Panayotis *et al*, 2015). How synaptic activity regulates gene expression is hotly debated as changes arise from rapid and transient increases in nuclear calcium, as well as from slow nuclear import of proteins by diffusion or motor-dependent transport processes (Panayotis *et al*, 2015). Long-distance protein transport from synapse to nucleus is thought to tailor broad calcium-based nuclear signaling and lead to synapse-specific regulation of function. Proteins located at the postsynaptic density such as Jacob, Abi-1, AIDA-1, and LAPSER1 translocate to the nucleus to regulate diverse nuclear functions including the activity of transcription factors such as CREB and Myc (Jordan & Kreutz, 2009; Kaushik *et al*, 2014). However, significantly less is known about presynapse-to-nucleus protein transport and its role in neuronal and synaptic function.

Formative studies in the marine gastropod *Aplysia* demonstrated that presynaptic events underlie new synapse formation, long-term facilitation, and behavior (Kandel, 2012; Martin *et al*, 1997a). MAPK shuttles along axons toward the nucleus upon synaptic activity (Martin *et al*, 1997b), and repair of nerve injury requires retrograde axonal transport of importin subunits (Panayotis *et al*, 2015). Recent work has shown that beta-amyloid peptides induce axonal synthesis and retrograde nuclear transport of ATF4/CREB2, which alters gene expression and causes neurodegeneration (Baleriola *et al*, 2014). However, whether direct shuttling of proteins between presynaptic termini and the nucleus plays a role in neuronal function remains unclear.

Ivanova and colleagues (Ivanova *et al*, 2015) show that the transcriptional co-repressor CtBP-1 (C-terminal-binding protein 1/brefeldin A-ADP-ribosylation) binds to presynaptic cytomatrix proteins Bassoon and Piccolo and shuttles between presynaptic terminals and nuclei following synaptic stimulation. In the absence of neuronal activity, CtBP-1 is enriched in the nucleus and represses the expression of immediate early genes (IEG) BDNF, Fos, and Arc. Neuronal activity, on the other hand, stabilizes CtBP-1 at presynaptic regions by increasing CtBP-1 binding to Bassoon, a process that requires nicotinamide adenine dinucleotide (NADH). Increased stability at presynaptic sites decreases CtBP-1 nuclear abundance and elevates transcription of its target genes. Together with RNAi-mediated knockdown experiments, the authors show that CtBP-1 regulates gene expression in presynaptic neurons following synaptic stimulation. Activity-dependent changes in nuclear and presynaptic CtBP-1 abundance were convincingly shown using fluorescence recovery after photobleaching (FRAP) and

photoactivatable GFP constructs. However, conclusive evidence of long-distance presynapse-to-nucleus transport will likely require additional experimentation, although such experiments may be technically challenging given the low number of presynaptic sites per neuron and their distance from the cellular soma. Regardless, these results represent an important first step toward demonstrating bidirectional protein transport between nuclei and presynaptic terminals and the role of this process in the regulation of gene expression.

Interestingly, the authors show that nucleocytoplasmic shuttling of CtBP-1 depends on cellular NADH abundance. Changes in NAD levels and activity are associated with Wallerian degeneration and are thought to underlie several neurodegenerative diseases (Coleman & Freeman, 2010). The ratio of oxidized to reduced NAD (NAD⁺/NADH) is altered following glycolysis and represents a sensitive measure of neuronal redox state and overall health. Activity-dependent regulation of neuronal metabolism is poorly understood but widely exploited in functional neuroimaging, such as positron-emission tomography (PET) and blood-oxygen-level-dependent contrast imaging (BOLD). Whether synaptic activity initiates downstream signaling cascades to regulate neuronal metabolic states remains to be determined. Therefore, understanding the implications of CtBP-1 nuclear import and export may shed light on molecular mechanisms linking synaptic activity to neuronal health and metabolism.

As with other studies in this field, technical and philosophical concerns remain and will ultimately need to be addressed for a comprehensive understanding of synapse-to-nucleus signaling. For example, concerns regarding the significance of small quantities of synaptic proteins in the nucleus are

¹ Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA

² Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, Bronx, NY, USA. E-mail: bryen.jordan@einstein.yu.edu

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Figure 1. Presynaptic activity regulates the intracellular distribution of CtBP-1.

Synaptic activity triggers CtBP-1 exit from the nucleus and transport to presynaptic terminals. This translocation depends on elevation of NADH levels, which is necessary to secure CtBP-1 binding to the presynaptic scaffolding molecules Bassoon and Piccolo. Absence of synaptic activity or inhibition of glycolysis causes nuclear accumulation of CtBP-1 and repression of its target genes among them Arc, Fos, and BDNF. Therefore, activity-dependent axonal transport of CtBP-1 regulates gene expression in presynaptic neurons.

exacerbated considering presynaptic zones are far less numerous than postsynaptic sites. However, a recent finding showed that activation of as few as three dendritic spines induced MAPK-dependent regulation of nuclear transcription factors, although these spines needed to be located in three distinct dendritic branches (Zhai *et al*, 2013). A second concern involves the vast distances traversed during anterograde/retrograde axonal transport. What are the advantages of localizing nuclear messengers at synapses given energetically expensive mechanisms for long-distance transport? Considering cellular efficiency and economy, speedier signaling through regenerating calcium waves could trigger nuclear export or import of somatically restricted proteins to regulate gene expression. There are a few reasonable explanations for long-range transport of proteins between synapses and nuclei: (1) the absence or presence of these proteins at synapses could have functional consequences *per se*, (2) slower protein-based nuclear signals could underlie an important and temporally distinct phase in the regulation of gene expression, or (3) protein messengers could retain coded information

regarding their synaptic origin to provide a mechanism for input-specific functional regulation. Here, the authors show that CtBP-1 accumulates at presynaptic terminals upon synaptic activity, although whether this is specific for active synapses is unclear. Accumulation at active synapses would raise the interesting possibility that CtBP-1 regulates the transport or local translation of newly synthesized transcripts as part of a synaptic tagging mechanism (Kandel, 2012). Whether CtBP-1 plays a role in the regulation of transcript metabolism remains to be determined.

The work of Ivanova *et al* (2015) highlights the important principle that both presynaptic and postsynaptic neurons undergo regulation of gene expression following synaptic stimulation. How and whether pre- and postsynaptic signaling cooperatively orchestrate gene expression to regulate synaptic plasticity is unknown. How does regulating gene expression in presynaptic neurons affect synaptic function? Do resulting transcripts modulate postsynaptic or presynaptic properties? These are essential questions that will need to be addressed in the future. It is interesting that nucleocytoplasmic shuttling of CtBP-1 was

observed several hours following synaptic simulation. This time frame is at odds with the rapid expression of Arc, Fos, and BDNF observed in postsynaptic neurons, although little is known about the temporal dynamics of IEG expression in presynaptic neurons. Changes in gene expression have been shown to occur in both acute and chronic phases (Kandel, 2012; Kaushik *et al*, 2014; Panayotis *et al*, 2015), and the time frame presented by Ivanova and colleagues (Ivanova *et al*, 2015) is consistent with chronic regulation of neuronal function following synaptic activity. Overall, this work represents an important step toward elucidating mechanisms underlying presynapse-to-nucleus signaling and further confirms that activity-dependent nuclear signaling is complex and rich in diversity.

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