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BONLAC: A combinatorial proteomic technique to measure stimulus-induced translational profiles in brain slices

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ABSTRACT

Stimulus-triggered protein synthesis is critical for brain health and function. However, due to technical hurdles, *de novo* neuronal translation is predominantly studied in cultured cells, whereas electrophysiological and circuit analyses often are performed in brain slices. The different properties of these two experimental systems create an information gap about stimulus-induced alterations in the expression of new proteins in mature circuits. To address this, we adapted two existing techniques, BONCAT and SILAC, to a combined proteomic technique, BONLAC, for use in acute adult hippocampal slices. Using BDNF-induced protein synthesis as a proof of concept, we found alterations in expression of proteins involved in neurotransmission, trafficking, and cation binding that differed from those found in a similar screen in cultured neurons. Our results indicate important differences between cultured neurons and slices, and suggest that BONLAC could be used to dissect proteomic changes underlying synaptic events in adult circuits.

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1. Introduction

De novo protein synthesis is a requirement for long-lasting changes in neuronal function and the consolidation of memory (Costa-Mattioli et al., 2009; Richter and Klann, 2009). Because neurons are polarized and have upwards of 10,000 synapses, on-demand mRNA translation occurs both in soma and dendrites, which results in rapid and stimulus-specific protein expression (Hanus and Schuman, 2013). Translational homeostasis therefore represents a major point of control for protein abundance (Schwanhäusser et al., 2011), and is critical for proper brain function.

Interestingly, our knowledge of neuronal translational control is predominantly derived from dissociated primary cultures and immortalized cell lines. In contrast, most information on synaptic plasticity, such as long term potentiation (LTP) and long-term depression (LTD), are derived from acutely prepared brain slices. These two systems are intrinsically disparate. For example, cultures are a monolayer of largely homogenous, disaggregated individual neurons that form stochastic connections as opposed to the multi-layer and genetically-encoded spatial organization that exists in slices. In addition, cultured neurons and mature brain slices

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represent different developmental time points, and brain slices contain glia and endothelial cells, which influence the final circuit response of a slice and is absent in neuronal cultures. These fundamental differences between slices and neuronal culture likely influence the overall proteomic and signaling profiles in response to various types of stimulation. Although the adult hippocampal slice has been used extensively to characterize multiple forms of synaptic plasticity, to date there are limited examples of large-scale unbiased study of proteomic response to chemical and electrical triggers in a slice, highlighting a gap in our current knowledge.

Among the available profiling techniques, shotgun proteomics has emerged as the method of choice given its high-throughput approach (Liao et al., 2009). Recently, bioorthogonal noncanonical amino acid tagging (BONCAT, Supplementary Fig. 1A (Dieterich et al., 2006)) and stable isotope labeling by amino acid in cell culture (SILAC, Supplementary Fig. 1B (Ong et al., 2002; Spellman et al., 2008)) were introduced to selectively isolate and quantify *de novo* proteins synthesized within a specified temporal window. BONCAT involves the tagging of nascent peptides with a methionine analog, which then is detected using a tagged-alkyne probe by a Huisgen chemistry reaction called cycloaddition (Link et al., 2007). SILAC employs isotopically-labeled, differentially weighted (heavy or medium) arginine and lysine that are incorporated into newly synthesized proteins to provide a mass spectrometry (MS) profile within the time frame of labeling (Liao et al., 2009). These techniques have been used in comparative proteomic analysis of protein synthesis in several cell culture systems, including cell lines that have higher protein synthesis rates than non-dividing cells (Howden et al., 2013; Selbach et al., 2008; Zhang et al., 2014). However, neither of these approaches either singly or in combination have been modified to measure the expression of newly synthesized proteins in an intact brain circuit.

We sought to adapt both BONCAT and SILAC to a multi-layered slice preparation to follow ongoing proteomic changes. As a standard for comparison, we used the well-studied neurotrophic factor, Brain-derived neurotrophic factor (BDNF), which has been shown to trigger neuronal protein synthesis, including local translation in cultured neurons (Aakalu et al., 2001; Liao et al., 2007; Dieterich et al., 2010). In slices, BDNF is mostly utilized to facilitate protein-synthesis dependent LTP (Figurov et al., 2006; Kang and Schuman, 1995; Ying et al., 2002; Kovalchuk et al., 2002; Kramár et al., 2004). We began by modifying the existing predominantly culture-based BONCAT and fluorescent non-canonical amino acid tagging (FUNCAT)-based protocols to study the response of a multi-layered slice to changes in protein synthesis by western blotting and immunofluorescence respectively. We then performed quantitative proteomics to identify and quantify the newly synthesized proteins using combinatorial approach of SILAC and BONCAT, which we have termed BONLAC, and then screened for changes in protein abundance of selected candidates. We finally compared the slice data to a similar BONLAC screen in cultured neurons, where we found that though candidates in slices and cultured neurons diverged appreciably, the cultured neuron data was consistent with previous reports suggesting inherent differences in proteomic response between the two systems. In summary, we demonstrate that BONLAC is a relatively easy and fast approach that is applicable to monitoring proteomic changes in response to a range of stimuli in intact brain slices.

2. Results

2.1. BONCAT and FUNCAT detect increases in protein synthesis in multiple layers of the hippocampal slice

BONCAT has been shown to detect neuronal translation in cultures previously (Dieterich et al., 2010), but required standardization for application in slices. This involved determining the incubation parameters of the methionine analog azido-homoalanine (AHA) and characterizing the specificity of the AHA-alkyne signal in a multi-layered tissue preparation. The standard incubation and manipulation medium for acute brain slices is artificial cerebrospinal fluid (ACSF), which does not supplement amino acids to the tissue as is done in culture media. Thus, slices probably retain an internal store of amino acids for several hours after dissection, the dynamics of which could modify the incorporation of the AHA into the available pool of amino acids. In addition, mammalian brains contain endogenous proteins that are post-translationally modified to contain biotin, which may interfere with signal detection. We tested a range of AHA concentrations from 0.5 mM to 3 mM, based on previous reports, and incubated slices with AHA for 1–5 h after recovery (see Methods). We found an optimal dose and timeframe for the application of BONCAT to slices at 1 mM AHA incubated for 3 h (see Supplementary Fig. S2A). To test the specificity of the BONCAT signal, we stimulated protein synthesis by treating slices with 1 μM Insulin for 10 min and pre-incubated slices with 40 μM anisomycin to abrogate translation. We observed robust increase in AHA signal in the insulin-treated slices, and almost entirely abrogated signal in the anisomycin-treated samples (Supplementary Fig. S2B). We noted the presence of two bands that appear consistently on all western blots and likely are signal of previously synthesized proteins complexed with biotin that did not change with AHA dose or any treatment (Supplementary Fig. S2A-B, white arrows). We also adapted the BONCAT-based biotin–avidin precipitation protocol to isolate biotin-alkyne labeled nascent proteins from these lysates and found a complete washout of signal in the anisomycin-treated lanes (Supplementary Fig. S2B, avidin pull-down). These results indicate that BONCAT is sensitive to dynamic alterations in translation in slices, such as insulin-induced protein synthesis, and that the AHA-alkyne-biotin detection method is largely capturing newly synthesized proteins within a given temporal window.

After establishing that BONCAT could detect short-term changes in protein synthesis, we then sought to examine the proteomic response to brain-derived neurotrophic factor (BDNF), a plasticity-inducing growth factor that has been shown to facilitate LTP (Figurov et al., 2006; Kang and Schuman, 1995; Ying et al., 2002; Kovalchuk et al., 2002; Kramár et al., 2004). We incubated hippocampal slices with 25 ng/ml BDNF for 1 h to detect elevated translation by BONCAT. After treatment of slices with BDNF for 1 h we noted a significant increase in AHA incorporation in hippocampal lysates (Fig. 1B–C) that was correlated with increased phosphorylation of BDNF receptor tyrosine kinase receptor B (TrkB) (Supplementary Fig. S2A). Thus, BONCAT is able to detect specific changes in protein synthesis driven by a biological stimulus by western blot in slices.

We proceeded to determine the spatial and regional pattern of the translational response to BDNF treatment in hippocampal slices. For this we independently derived a protocol similar to Dieterich et al. (2010) for use of FUNCAT to brain slices, where AHA is detected using an alkyne tagged to Alexa 488 and subsequent fluorescence imaging. BDNF treatment of 1 h resulted in an increase in fluorescence in area CA1 and the dentate gyrus (DG) compared to control slices, which was present in both the soma and the dendrites of the neurons (Fig. 1D–E). In addition, there was a greater BDNF-induced change in new protein synthesis in the DG compared to area CA1 (Fig. 1D–E). Because slices are multi-layered, it was unclear whether the BDNF-mediated increase in translation was either restricted to specific layers or was uniform throughout the slice. As electrophysiological recordings are best obtained from internal layers of a slice, it was important to establish the spatial