Study of protein and RNA in dendritic spines using multi-isotope imaging mass spectrometry (MIMS)

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ABSTRACT:
The classical view of neuronal protein synthesis is that proteins are made in the cell body and then transported to their functional sites in the dendrites and the dendritic spines. Indirect evidence, however, suggest that protein synthesis can directly occur in the distal dendrites, far from the cell body. We are developing protocols for dual labeling of RNA and proteins using 15N-uridine and 15O or 13C-leucine pulse chase in cultured neurons to identify and localize both protein synthesis and fate of newly synthesized proteins. Primary cultures of hippocampal cells from embryonic mice are established on Si chips. Culture media is supplemented with 15N-Uridine and 13C-leucine (or 13C-leucine) individually and in combination starting at a series of different days in culture. MIMS analysis reveals RNA by the 15N signal and synthesized proteins by the 15O signal. Quantitative and spatial analysis are used to assess the time course and localization of protein synthesis as well as the fate of synthesized proteins. Pilot experiments show discrete localization of both RNA and newly synthesized proteins in dendrites, close to dendritic spines. We have for the first time directly imaged and measured the production of proteins at the subcellular level in the neuronal dendrites, close to the functional sites, the dendritic spines. This will open a powerful way to study neural growth and synapse plasticity in health and disease. The highest technical challenge will be to well preserve the dendritic spines. This will open a powerful way to study neural growth and synapse plasticity in health and disease. An understanding of the dendritic protein synthesis machinery will open pathways for development of new pharmacology and new therapeutic strategies for psychiatric disorders.

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CONCLUSION:
The first time, we directly measured protein synthesis and RNA presence in dendrites of cultured embryonic mouse hippocampal neurons. This will be applied to study Long Term Potentiation and Long Term Depression of synaptic activity. This will be applied to study single gene disorder that intersect with Autism Disorder Spectrum genetic defect affecting the brain like in the fragile X syndrome. Particularly mutations in FMR2, the major cause of inherited ASD, which results in the absence of Fragile X mental retardation protein (FMRP), a major regulator of activity dependent synaptic protein synthesis. This will be extended to in vivo study of animal models.

Figure 1.

Figure 2.

Figure 3.

Figure 4.