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Upregulation of eIF4E mRNAs in the Dendrites of Hippocampal Neurons by KCl Treatment

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Activity-dependent local dendritic translation in CNS neurons plays important roles in synapse-specific provision of proteins necessary for strengthening of synaptic connections. Availability of eukaryotic translation initiation factor 4E (eIF4E), an mRNA 5'-cap-binding protein, is the major rate-limiting factor for protein synthesis. Fluorescence in situ hybridization (FISH) revealed that treatment of the neurons with KCl increases very significantly ($p>0.01$) in dendritically localized eIF4E mRNAs. By combining FISH with immunocytochemistry (IC), we further showed that KCl treatment increases very significantly ($p>0.01$) in the PSD95-associated eIF4E mRNA punctae. These results demonstrate an activity-dependent increase of eIF4E mRNA at synaptic sites.

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Combined FISH (Fluorescence *in situ* hybridization) and Immunocytochemistry

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In this manuscript, we report a simplified but robust protocol that allows immunocytochemical localization of proteins after ISH. In this protocol, we fix cultured cortical or hippocampal neurons with 4% paraformaldehyde (PFA), rinse briefly in PBS, and then further fix the cells with -20°C methanol. Our method has several major advantages over previously described ones in that (1) it is just consecutive routine fixation procedures, (2) it does not require any special alteration to the fixation procedures, and (3) it can be used with antibodies that are compatible with either MeOH- or PFA-fixed target proteins.