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DISSERTATION SUMMARY

In vitro analysis of the intracellular distribution of different calcium-binding proteins and their corresponding mRNAs

Szilvia Várszegi

Department of Zoology and Cell Biology, University of Szeged, Szeged, Hungary

Calmodulin (CaM) is a multifunctional, highly conserved Ca^{2+}-binding protein particularly abundant in neural tissue. It is involved in signalling pathways that regulate many crucial cellular processes. In the central nervous system, a vast majority of CaM is synthesized by neurons, whereas the glial CaM expression is much weaker. In higher vertebrates three CaM genes are collectively transcribed into at least eight different mRNAs, although each mRNA codes for the same protein. Messenger RNAs transcribed from all three CaM genes are significantly more abundant in dendrite-rich regions than in axon-rich regions of rat brain (Palfi et al. 2002). In order to investigate the intracellular distribution of CaM mRNAs in detail we carried out CaM gene-specific in situ hybridization cytochemistry (ISH) with digoxigenin-labeled riboprobes on rat primary hippocampal cultures. The perykaryon was heavily stained in neurons and strong dendritic mRNA targeting was detected for all three CaM genes. The color labeling exhibited a punctate distribution, suggesting that CaM mRNAs are transported in RNA granules. To identify the cells with nondetectable CaM expression, glia-specific S100 immunocytochemistry was carried out after the ISH protocol. The vast majority of the CaM negative cells proved to be S100 positive, whereas the neurons expressing the CaM genes did not express the S100 marker (Kortvely et al. 2003).

The number of known dendritically targeted mRNA species in mammalian neurons is growing and evidence indicates that these mRNAs are translated there. Many localized mRNAs contain specific targeting element in their 3'-untranslated region (3'-UTR). The mRNA of the Ca^{2+}/CaM-dependent protein kinase II α (CaMKIIα), which is one of the most important target protein of CaM, is also localized dendritically, and its 3'-UTR can mediate this localization (Rook et al. 2000). A cis-acting control element, the cytoplasmic polyadenilation element (CPE) can facilitate mRNA transport to dendrites (Huang et al. 2003). Since the 3'-UTR of the CaMI and CaMKIIα mRNAs contain CPEs, we assume that these mRNAs are cotransported to dendrites. To test our hypothesis, fluorescent in situ hybridization cytochemistry was carried out with biotin- and digoxigenin-labeled riboprobes for CaMI and CaMKIIα mRNA, respectively, on B104 neuroblastoma and cultured primary hippocampal cells. Our preliminary experiments indicate that these two mRNAs are colocalized in dendrites, raising the possibility of their local synaptic activity-dependent cotranslation.

References

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Supervisor: Károly Gulya
E-mail: nyulszi@freemail.hu