

DISSERTATION SUMMARY**Calmodulin gene expression in central nervous system areas of low calmodulin mRNA abundance**

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The calmodulin (CaM) protein plays a complex role in the regulation of the different cytoplasmic processes in general, and also (because of its paramount role in the neuronal signalization processes) in the nervous system as a whole in particular. While this protein is often present in medium-to-high concentrations in the nervous tissue, there are other regions (the spinal cord and the retina) that contain CaM only in minute quantities, and the amounts of the different CaM transcripts in these regions are also low. Our knowledge on the CaM gene expression in the different cell types of the spinal cord and the retina is sparse, mainly in consequence of their low CaM mRNA contents. Thus, during the initial stage of our research, we developed a sensitive color in situ hybridization method (Kovacs and Gulya 2001), employing a slightly alkaline pH (pH 8.0) in the hybridization mixture, which is sensitive enough to detect low-abundance CaM transcripts in areas of the central nervous system (the white matter of the spinal cord and the retina) that may also have high lipid contents (the white matter areas of the spinal cord).

On the basis of their morphology, we detected two distinct cell types that express CaM in the white matter area of the rat spinal cord (Palfi et al. 2002; Kovacs and Gulya 2002). The medium-sized, astrocyte-like cells residing mainly in the dorsal column of the white matter displayed differential CaM expression: the CaM I mRNA content was highest, followed by the CaM III and CaM II contents in these cells. The CaM gene expression of the oligodendrocytes (in both the dorsal and the lateral columns) was less differentiated, although the CaM I mRNA content was slightly higher than that of the others. Our results indicate that 1) the CaM expression profile of the spinal cord is richer and more complex than previously thought on the basis of conventional radioactive in situ hybridization techniques, and 2) when a method that is sufficiently sensitive was used, more cell types could be demonstrated to express CaM mRNAs; thus, in spite of their lower CaM expression and their lipid-rich environment, glial cells could also be visualized.

The retina is a tissue with low lipid content, where the conventional in situ hybridization techniques with relatively low sensitivity did not detect any CaM-expressing cells, in

spite of the fact that the presence of the protein was previously demonstrated by immunohistochemical techniques. Our sensitive in situ hybridization technique revealed the presence of CaM mRNA populations in the adult rat retina, and we concluded that the expression levels of the different CaM genes were almost identical (Kovacs and Gulya 2003). Although the layer-specific distributions of these genes are strikingly similar, there are major differences in CaM expression within the different retinal layers. The strongest signals for all CaM mRNAs were demonstrated in the ganglion cell layer and the inner nuclear layer. Intermediate signal intensities for all CaM genes were detected in the inner and outer plexiform layers, within the vicinity of the outer limiting membrane and in the pigment epithelium. Very low specific signals were characteristic in the outer nuclear layer and the photoreceptor inner segment layer, while no specific hybridization signal was observed in the photoreceptor outer segment layer.

In summary, our sensitive in situ hybridization technique was able to detect low-abundance CaM transcripts in the white matter of the rat spinal cord and the neural retina of the adult rat (Kovacs 2003). Our results contribute to a better understanding of the functional role(s) of the CaM protein and also of the CaM gene expression in these regions.

References

- Kovacs B, Gulya K (2001) A color in situ hybridization method with improved sensitivity for the detection of low-abundance mRNAs. *Acta Biol Szeged* 45:75-77.
- Palfi A, Kortvely E, Fekete E, Kovacs B, Varszegi S, Gulya K (2002) Differential calmodulin gene expression in the rodent brain. *Life Sci* 70:2829-2855.
- Kovacs B, Gulya K (2002) Differential expression of multiple calmodulin genes in cells of the white matter of the rat spinal cord. *Mol Brain Res* 102:28-34.
- Szigeti C, Kovacs B, Kortvely E, Gulya K (2003) Comparison of treatment regimens to sensitize in situ hybridization for low-abundance calmodulin transcripts in the white matter of the rat spinal cord. *Acta Biol Szeged* 47:1-6.
- Kovacs B (2003) Calmodulin gene expression in central nervous system areas of low calmodulin abundance. *Acta Biol Szeged* 47:71.
- Kovacs B, Gulya K (2003) Calmodulin gene expression in the neural retina of the adult rat. *Life Sci* 73:3213-3224.

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