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The role of perinuclear mAKAP signalosome in the regulation of NFAT function in primary hippocampal neurons

Joanna Mackiewicz, joanna.mackiewicz1@stud.umed.lodz.pl
Supervisors: Tomasz Boczek, PhD; Malwina Lisek, PhD
Department of Molecular Neurochemistry, Medical University, Lodz, Poland

INTRODUCTION

Muscle A-kinase anchoring protein (mAKAP) is a scaffold protein that exists in two alternatively spliced forms: mAKAPα and mAKAPβ. The longer form - mAKAPα is preferentially expressed in the brain. mAKAPβ is a shorter form of anchoring protein that lacks the first 244 amino acids and is principally expressed in the heart. The function of neuronal mAKAPα has not been well-characterized. It has been shown that mAKAP binds a large number of enzymes involved in cell signaling, including calcium-dependent phosphatase calcineurin (CaN) and the activator of nuclear factor of activated T-cells (NFAT). In humans, the NFAT family comprises of five transcription factors named as follows: NFAT1 (NFATc2), NFAT2 (NFATc1), NFAT3 (NFATc4),NFAT4 (NFATc3) and NFAT5. Upon activation by CaN, NFATs translocate from cytosol to the nucleus and regulate their target genes that are involved in neuronal axon outgrowth, synaptic plasticity, and survival. Despite the critical role of NFAT-dependent transcription in neurons, it is not known how the activity of these transcription factors is regulated.

RESEARCH HYPOTHESIS

By binding transcription factors of the NFAT family, mAKAP signalosome is hypothesized to regulate NFAT nuclear translocation and NFAT-dependent transcription critical for neuronal survival and axonal outgrowth.

METHODOLOGY

Investigation of mAKAPα expression and mAKAPα-NFATc4 interaction in primary hippocampal neurons were carried out using Western Blot and co-immunoprecipitation techniques. Neuronal extension was analyzed based on fluorescence images of co-transfected cells.

RESULTS

- mAKAPα is expressed in primary rat hippocamal neurons (Fig. 1A),
- mAKAPα binds NFATc4 in primary rat hippocampal neurons (Fig.1B),
- interaction between mAKAPα and NFATc4 is enhanced following KCl stimulation indicating its Ca^{2+}-dependence (Fig. 2A),
- recruitment of NFATc4 to mAKAPα complex is dependent on CaN activity (Fig. 2B),
- overexpression of NFATc4 does not affect axonal outgrowth in the presence or absence of KCl (Fig. 3 and 4),
- the perinuclear calcineurin is required for KCl-dependent axon elongation (Fig. 5 and 6),
- shRNA against mAKAPα decreases neurite extension in the presence of KCL (Fig. 7 and 8).

How mAKAPα - NFATc4 interaction is regulated in neurons?

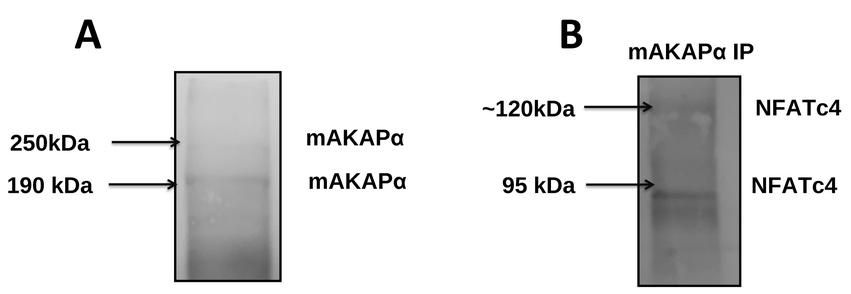

Figure 1. Western blot demonstrated mAKAPα expression (A) and mAKAPα - NFATc4 interaction (B) in primary rat hippocampal culture.

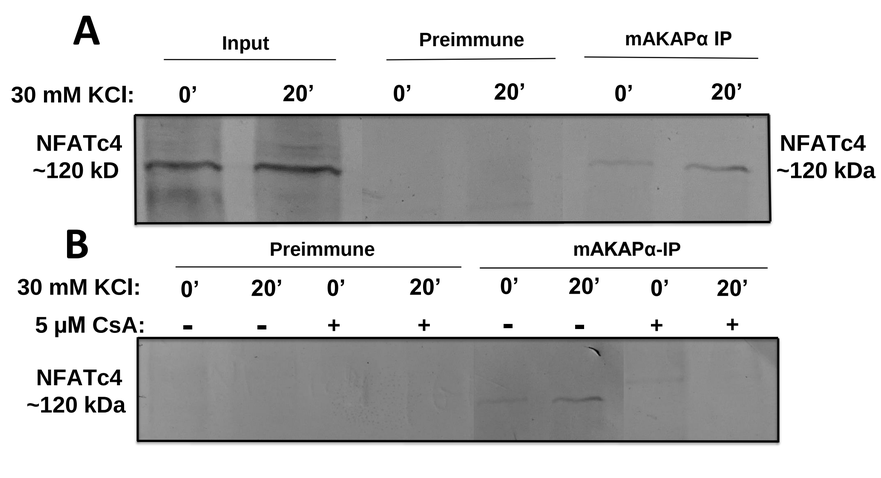

Figure 2. Binding of NFAT to mAKAPα is enhanced in the presence of KCl (A) but abolished when CaN activity is inhibited using cyclosporin A (CsA) (B).

The importance of NFATc4 in the growth of axons of primary neurons

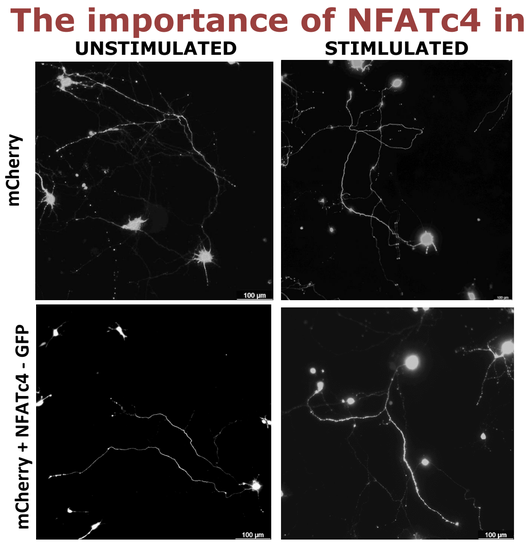

Figure 3. Representative images of hippocampal neurons expressing mCherry and both mCherry and NFATc4-GFP in basal (unstimulated) conditions and following KC stimulation. Images were acquired with Leica DMi8 fluorescence microscope by 20x objective tile scan and processed with Image J. Scale bar - 100 μm.

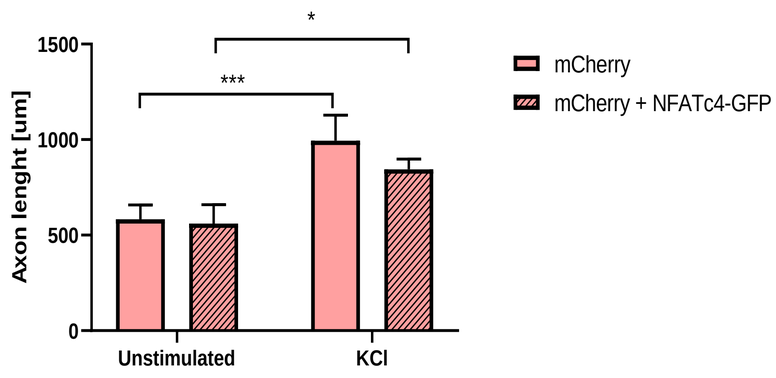

Figure 4. The length of neurons expressing mCherry and both mCherry and NFATc4-FP. Live-cell images were performed under resting condition and following KCl stimulation. Each value represents the mean value ± S.D, each experiment was carried out in triplicate. *p<0.05,***p<0.001.

The importance of perinuclear calcineurin in the growth of axons of primary neurons

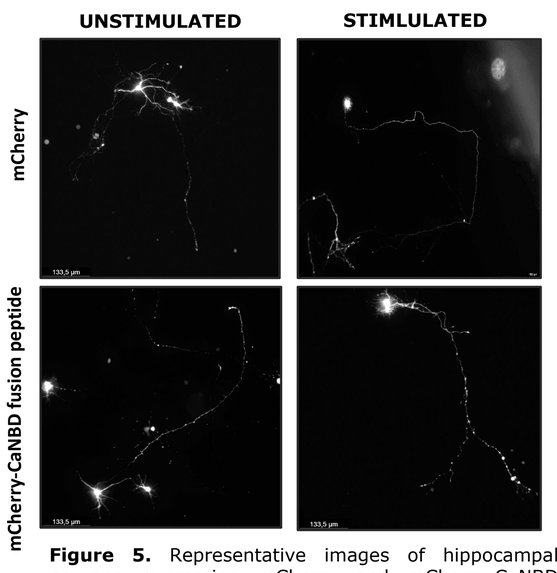

Figure 5. Representative images of hippocampal neurons expressing mCherry and mCherry-CaNBD fusion peptide (unstimulated) conditions and following KCl stimulation. Images were acquired with Leica DMi8 fluorescence microscope by 20x objective tile scan and processed with Image J. Scale bar – 133,5 μm and 253,8 μm.

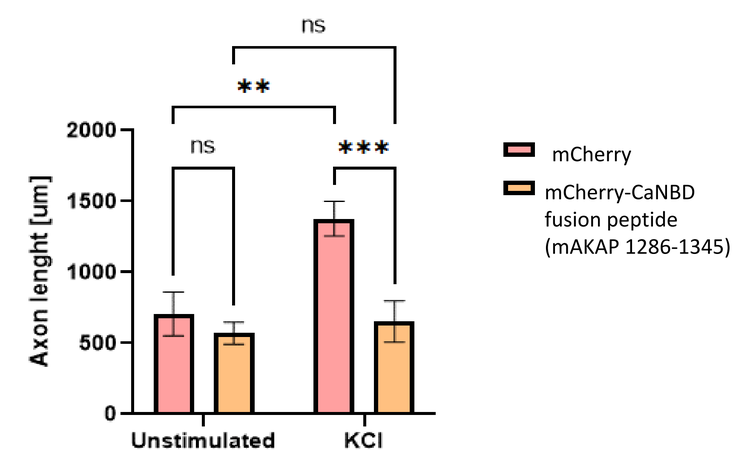

Figure 6. The length of neurons expressing mCherry and mCherry-CaNBD fusion peptide. Live-cell images were performed under resting condition and following KCl stimulation. Each value represents the mean value ± S.D, each experiment was carried out in triplicate. **p<0.01,***p<0.001.

The importance of mAKAPα in the growth of axons of primary neurons

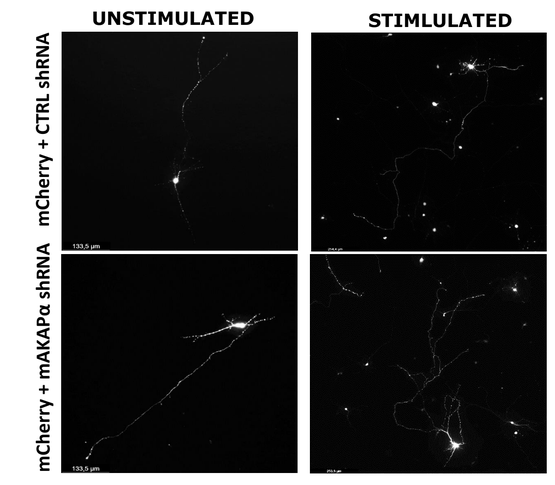

Figure 7. Representative images of hippocampal neurons expressing both mCherry and shRNA control (CTRL shRNA) or mCherry and shRNA mAKAPα (unstimulated) conditions and following KCl stimulation. Images were acquired with Leica DMi8 fluorescence microscope by 20x objective tile scan and processed with Image J. Scale bar – 133,5 μm and 253,8 μm.

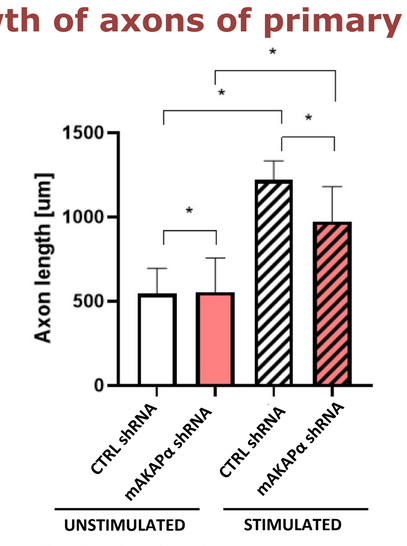

Figure 8. The length of neurons expressing control shRNA (CTRL shRNA) and mAKAPα shRNA. Live-cell images were performed under resting condition and following KCl stimulation. Each value represents the mean value ± S.D, each experiment was carried out in triplicate. *p<0.05

CONCLUSIONS

The association of NFATc4 with mAKAP signalosome is enhanced during neuronal depolarization and depends on the activity of calcineurin. Thus, mAKAP located in the perinuclear space may be a critical point for NFATc4 activation and nuclear translocation. In this process, mAKAP-dependent NFATc4 dephosphorylation seems to play an important role. Moreover, our results indicate a key role of calcineurin/mAKAP interaction for neuronal extension.