MEDICAL UNIVERSITY OF LODZ

International Doctoral School
Medical University of Lodz
Joanna Mackiewicz

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

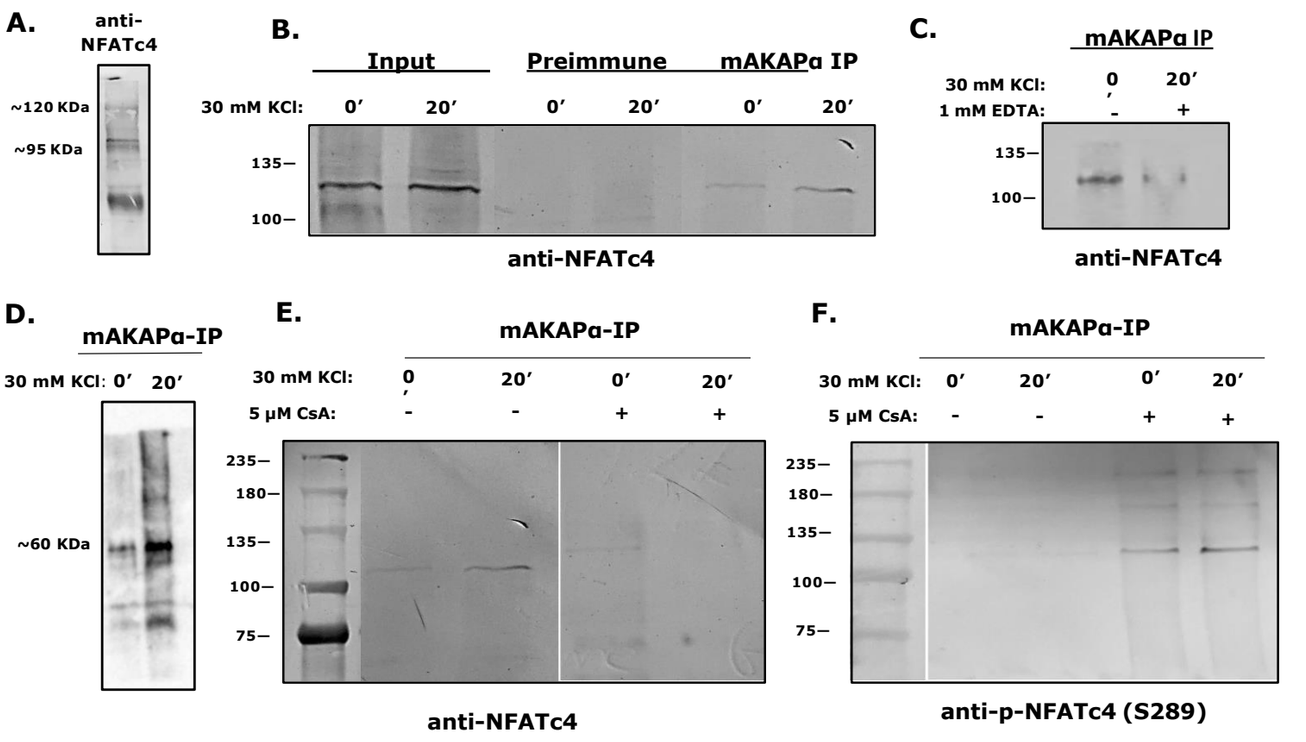
Muscle A-kinase anchoring protein (mAKAP) is a scaffold protein that exists in two alternatively spliced forms: mAKAPα and mAKAPβ. The longer form - mAKAPα is preferentially expressed in the brain. mAKAPβ is a shorter form of anchoring protein that lacks the first 244 amino acids and is principally expressed 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) which is an upstream activator of nuclear factor of activated T-cells (NFAT). To verify the importance of mAKAP-mediated CaN scaffolding, we have constructed mCherry-fused anchoring disrupting peptide corresponding to 1286-1345 of mAKAP. The expression of the peptide below the saturation level, disrupted KCl-stimulated axonal elongation of hippocampal neurons compared to mCherry-only labelled neurons without affecting the survival Silencing of mAKAP with shRNA resulted in similar effect to mCherry-1286-1354 pointing out mAKAP importance for CaN-dependent axonal outgrowth. Interestingly, neither mAKAP shRNA nor mCherry-1286-1354 were able to affect neuronal growth in the absence of depolarizing stimulus. This observation corresponded to enhanced CaN binding to mAKAP following neuronal depolarization and subsequent promotion of NFATc4-mAKAP association. Testing for the site of NFATc interaction, we found NFATc binding site location in N-terminal domain of mAKAP (amino acids 1-196). However, in contrast to mCherry-1286-1354, overexpression of NFATc4 did not affect the axonal length of depolarized neurons indicating a mechanism independent from NFATc.

RESEARCH HYPOTHESIS

mAKAP signalosome is hypothesized to regulate NFATc4 activty critical for neuronal survival and axonal outgrowth

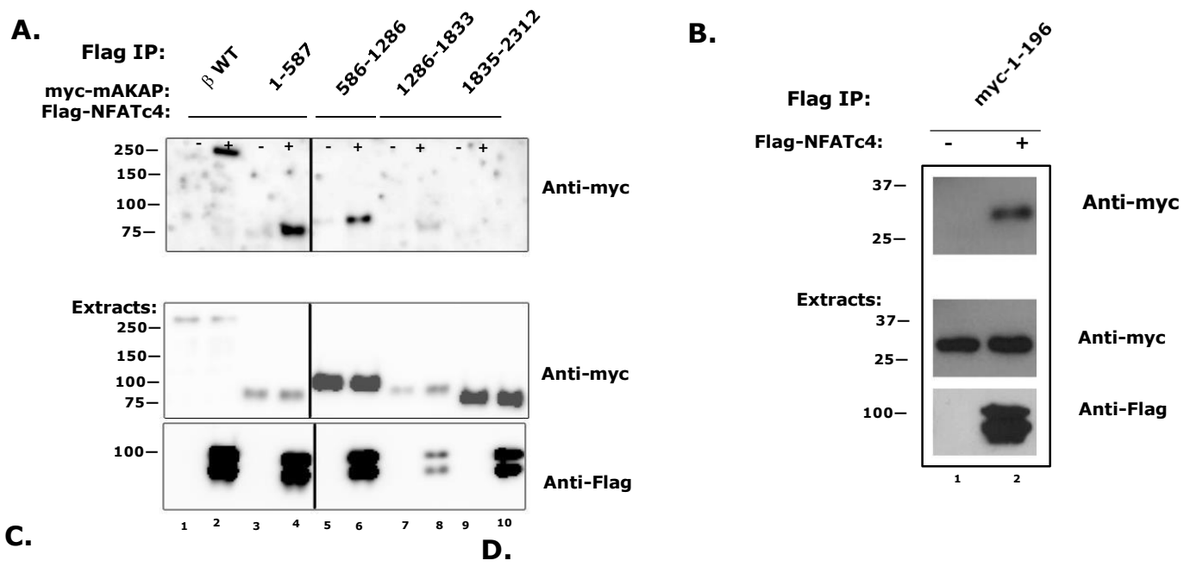
RESULTS

mAKAPα interacts with NFATc4 in Ca^{2+}- and CaN-dependnet manner



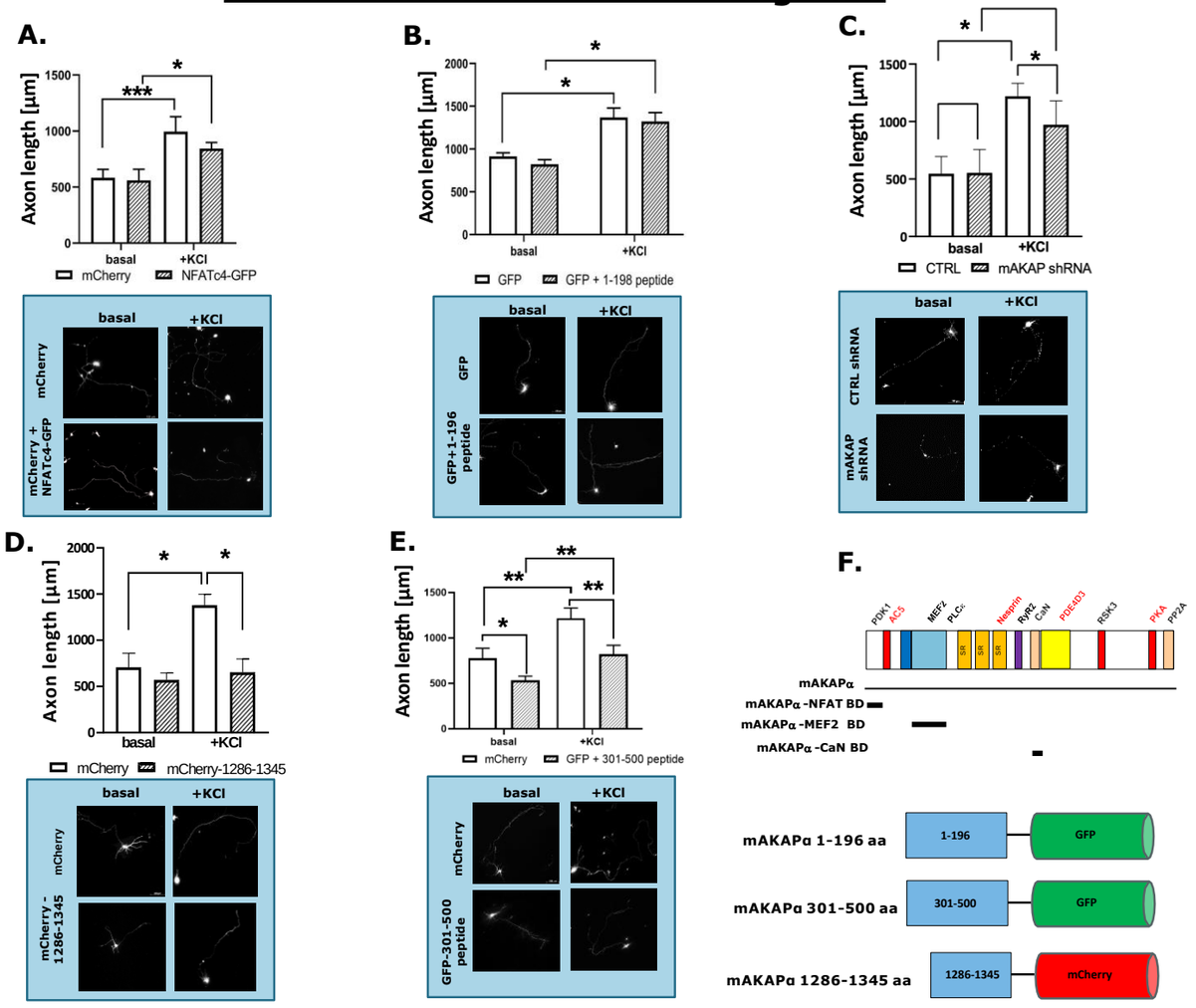
(A) NFATc4 immunoprecipitates with mAKAPα. (B) mAKAPα/NFATc4 interaction is enhanced in the presence of KCl and (C) inhibited by EDTA or (E) cyclosporine. (D) mAKAPα/CaN interaction is enhanced in the presence of KCl. (E) S289-phosphorylated NFATc4 does not immunoprecipitate with mAKAPα suggesting preferential binding upon CaN dephosphorylation.

Mapping NFAT binding site in the N-terminus of the scaffold



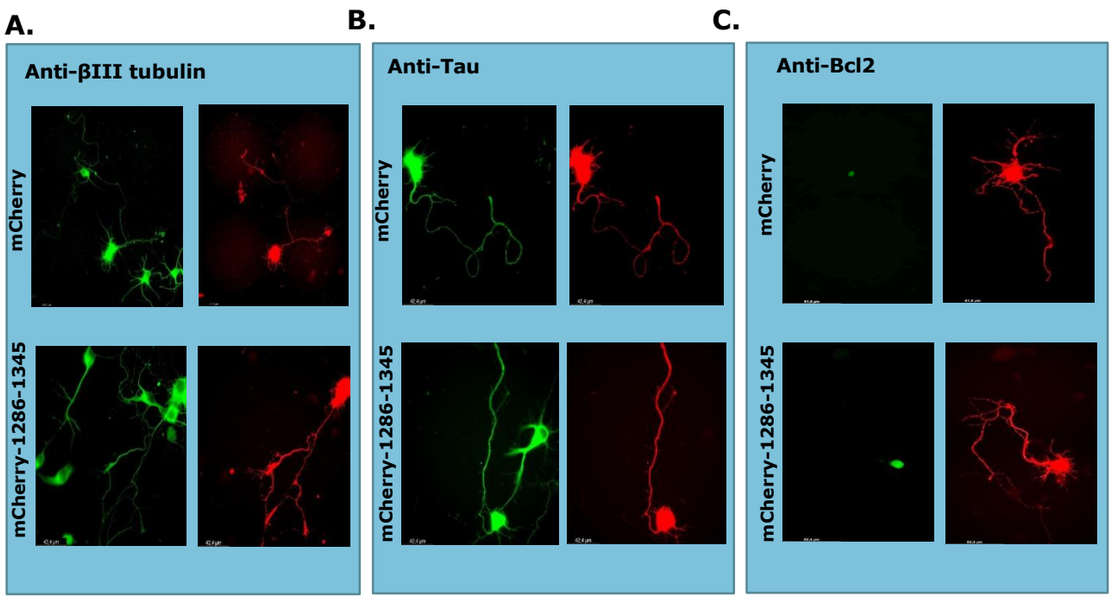
(A) NFATc4 interacts with both mAKAPα and β. (B) Mapping NFATc4-mAKAPα interaction. (C) NFATc4-mAKAP interaction in the presence of exogenous PKA and GSK-3β kinases. (D) Minimal binding fragment for NFATc4.

The effect of overexpression of NFATc4, mAKAPα itself, mAKAPα-NFAT,mAKAPα-CaN and mAKAPα-MEF2 interaction for KCl-stimulated axonal elongation



(A) Neurite outgrowth in the presence of mCherry or both mCherry and NFATc4-GFP. (B) Length of neurons expressing GFP or GFP-1-196 peptide. (C) Neurite outgrowth following mAKAPα silencing. (D) Neurite outgrowth in the presence of mCherry-1286-1345. (E) Neurite outgrowth in the presence of GFP-301-500 peptide. Scal bar 100 μm. (F) Construction of displacing peptides. ***p<0.001,**p<0,01, *p<0.05.

mAKAPα-CaN interaction in the survival of neurons



(A) βIII-tubulin, (B) Tau and (C) BCl2 staining in the presence of KCl. Scale 42.4 μm.

CONCLUSIONS

Our data strongly suggest that mAKAP located in the perinuclear space may serve as a nodel point for the control of CaN and MEF2 action in the outgrowth of hippocampal neurons. Furthermore these data also illustrate how scaffold proteins organizing localized signaling complex provide the molecular architecure for signal transduction networks regulating neuronal phenotype