INTERACTION OF MITOCHONDRIAL PROTEIN NIPSNAP1 WITH AMYLOID PRECURSOR PROTEIN (APP)

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ABSTRACT

Mutations in amyloid precursor protein (APP) have been linked to Alzheimer's disease (AD), a progressive neurodegenerative disease associated with significant neuronal loss and disruption of energy metabolism in the brain. However, the physiological role of APP and its family members is still unclear. To gain insights into APP function, we used a proteomic approach to identify APP interacting proteins. Previously, we reported for the first time a direct interaction between the C-terminal region of APP family proteins and ubiquitous mitochondrial creatine kinase (uMtCK, Li et al., 2006 Mol Cell Neurosci. 31(2):263-272). Here, we describe an interaction between APP and a novel protein, 4-nitrophenylphosphatase domain and non-neuronal SNAP25- like protein homolog 1 (NIPSNAP1). This interaction was confirmed both in cell culture and in mouse brain. In addition, we provide experimental evidence to demonstrate NIPSNAP1 is targeted to the mitochondria via its N-terminal mitochondrial targeting sequence. Interaction of APP C-terminal peptide stabilized NIPSNAP1 pre-protein, in a manner similar to our previous observations with uMtCK. However, full length APP, which is membrane bound, destabilized NIPSNAP1 mature protein. Our data suggest that APP may modulate protein targeting to the mitochondria, hence, may directly be involved in regulation of mitochondrial function.

Abbreviations: **APP**, Amyloid precursor protein; **uMtCK**, ubiquitous mitochondrial creatine kinase; **NIPSNAP1**, 4-nitrophenylphosphatase domain and non-neuronal SNAP25- like protein homolog 1; **IP**, Immunoprecipitation; **WB**, Western blot; **CytC**, Cytochrome C; **WT,** wildtype; **SWE**, Swedish mutant.

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Introduction

APP family of genes

	Genotype	Viability[1]
1	APP -/-	Viable and fertile
2	APLP1 -/-	Viable and fertile
3	APLP2 -/-	Viable and fertile
4	APLP2-/-, APP-/-	Perinatally lethal
5	APLP2-/-, APLP1-/-	Perinatally lethal
6	APLP1-/-, APP-/-	Viable and fertile

There is functional redundancy between APP family members

1. Heber et al., J Neurosci. 2000 Nov 1;20(21):7951-63. (Dr. Ulrike Muller group)

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OBJECTIVE OF THE STUDY

The C-terminal region is the most conserved region among APP family proteins

To identify proteins that interact with C-terminal of APP family of proteins by using molecular proteomics and also study the physiological and pathological significance of these interactions.

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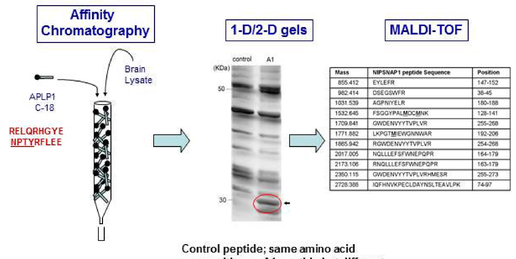
Identification of NIPSNAP1 in Proteomic Screen



Control peptide; same amino acid composition as A1 peptide but different sequence

The C-terminal 18 amino acid region of APLP1 specifically binds to a novel protein NIPSNAP1

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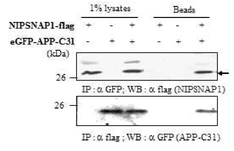
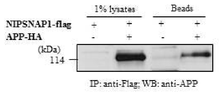
NIPSNAP1-APP Interaction : Validation in COS7 cells

Method: Transient transfection & Co-immunoprecipitation

NIPSNAP1 binding to APP-C31 peptide



NIPSNAP1 binding to full length APP



NIPSNAP1 interacts with APP in transiently transfected cell culture system

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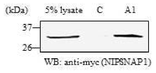
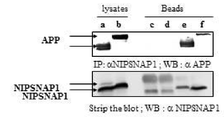
NIPSNAP1-APP Interaction : Validation in mouse brain lysates

NIPSNAP1 binding to APP-C18 peptide



Identification of NIPSNAP1 in complexes purified from mouse brain lysates using immobilized control (C) or APLP1 c-terminal 18 amino acid (A1) peptides.

NIPSNAP1 binding to full length APP



Adult mouse brain (**a,c,d & e**) and liver (**b & f**) lysates were incubated with immobilized affinity purified anti-NIPSNAP1 antibodies (**e & f**) or pre-immune sera (c) or flow through obtained during antibody purification (**d**).

APP exists in complex with NIPSNAP1 in mouse brain lysates.

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What is NIPSNAP1?

- Is expressed ubiquitously and may be involved in vesicular transport.
- Is Associated with postsynaptic density.
- NIPSNAP1 protein levels are increased during synaptic activity.
- NIPSNAP1 mRNA levels are increased 8-fold in Phenylketonuria mouse brains.
- NIPSNAP1 appears to play a role in synaptic plasticity and cognitive functions, including memory.

1. Seroussi et al., Gene. 1998 May 28;212(1):13-20.
2. Satoh et al., Genes Cells. 2002 Feb;7(2):187-97.
3. Surendren et al., Neurochem Int. 2005 Jun;46(8):595-9.

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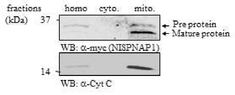
Sub-cellular localization of NIPSNAP1

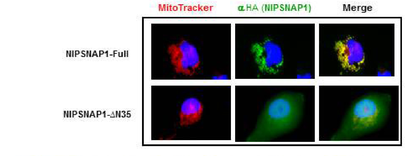
NIPSNAP1 contains a 36 a.a. N-terminal mitochondrial signal sequence:

MAPRLCIISAAARRLFTKPRPRAGDLAAAGAVRFYS

NIPSNAP1 is enriched in mitochondrial fractions:



NIPSNAP1 is co-localized with mitochondria:



NIPSNAP1 is targeted to the mitochondria using its N-terminal signal sequence

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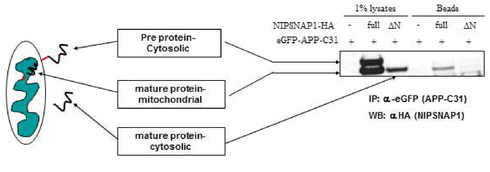
Sub-cellular localization of NIPSNAP1 : APP Binding

Method: Transient Transfection & Co-immunoprecipitation



Mitochondrial localization of NIPSNAP is necessary for APP Binding

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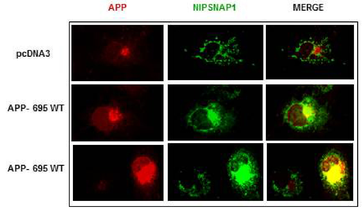
APP-NIPSNAP1 interaction : NIPSNAP1 Localization

Method: COS7 cells were co-transfected with APP and NIPSNAP1-HA and immuno-stained using anti-APP and anti-HA antibodies



Co-expression of APP changes the localization of NIPSNAP1

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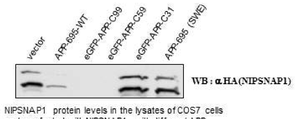
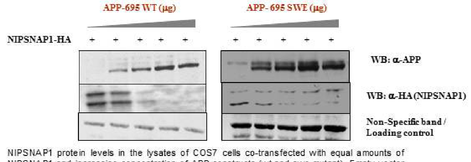
APP regulates NIPSNAP1 protein levels



NIPSNAP1 protein levels in the lysates of COS7 cells co-transfected with NIPSNAP1 with different APP constructs.



NIPSNAP1 protein levels in the lysates of COS7 cells co-transfected with equal amounts of NIPSNAP1 and increasing concentration of APP constructs (wt and swe mutant). Empty vector was used to keep the total plasmid amount constant.

Co-expression of wild type APP, but not the Swedish mutant of APP, decreases the NIPSNAP1 protein levels.

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CONCLUSIONS

- The C-terminal region of APP and its family members bind to NIPSNAP1.
- NIPSNAP1 is a mitochondrial protein.
- Mitochondrial localization of NIPSNAP1 is necessary for APP binding.
- APP interaction appears to change the localization of NIPSNAP1 and also decreases its protein levels.
- Co-expression of wild-type APP, but not the Swedish mutant APP, decreases NIPSNAP1 protein levels.
- The C-terminal 31 a.a. of APP stabilizes NIPSNAP1 while C-terminal 59 and 99 a.a peptides destabilize NIPSNAP1 protein.

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