DHHC8-mediated Paralemmin-1 Membrane Localization

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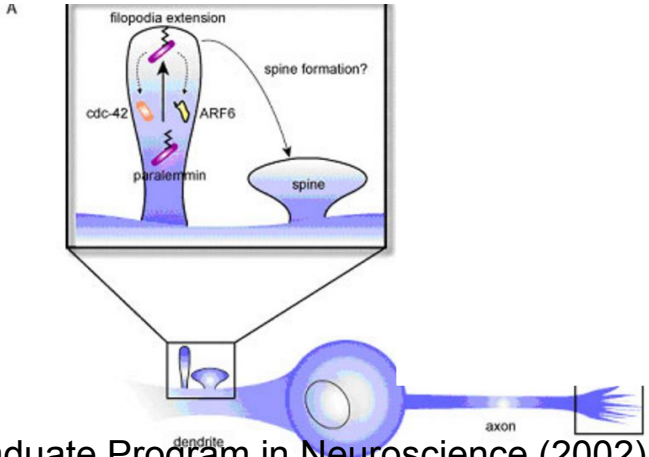
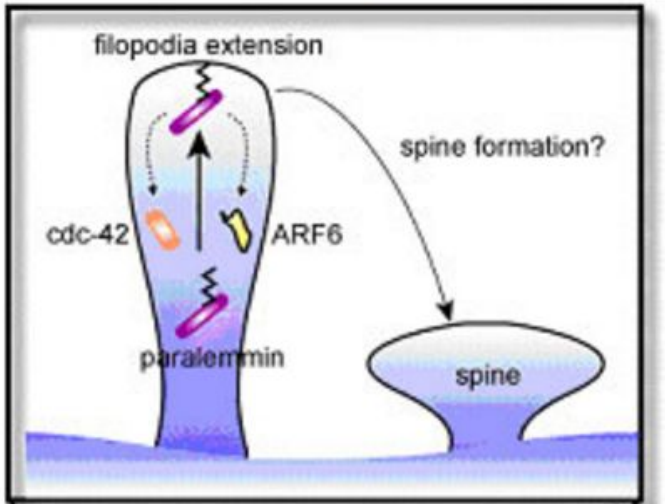
CAPS 200

Introduction: Paralemmin-1

- Important for filopodia formation and spine maturation
- Localizes in plasma membrane, anchored by lipid motif
- Translocation is activity regulated
- Increased density at membrane expands membrane

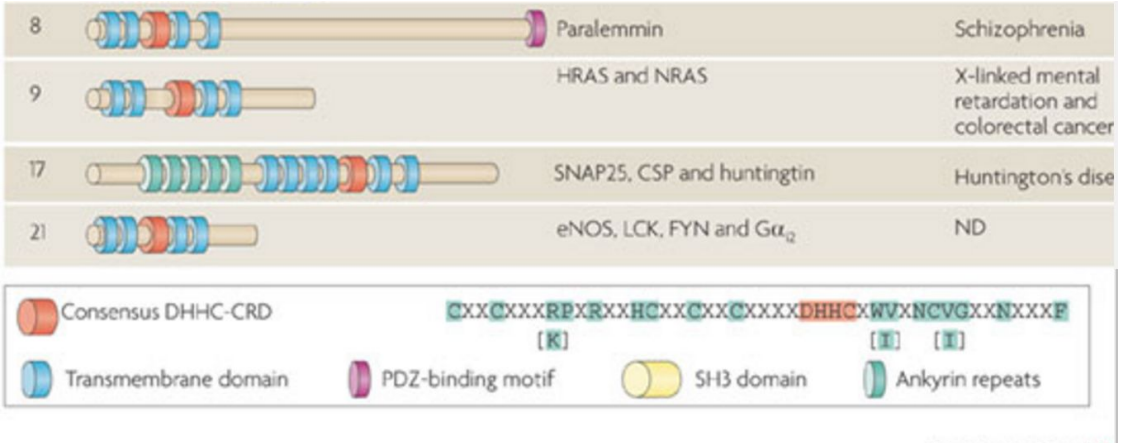


Graduate Program in Neuroscience (2002)



Introduction

- Paralemmin-1 is a substrate for DHHC8
- Addition of palmitate makes Paralemmin-1 more lipophilic
- DHHC8 palmitoylates Paralemmin-1 in response to activity



Fukata Y., & Fukata M (2010)

Question/Hypothesis/Specific Aims

Question:

- How does LTP cause an increase in the membrane localization of paralemmin-1?

Hypothesis:

- LTP causes DHHC8 to migrate and bind to paralemmin-1, causing the palmitoylation of paralemmin-1, allowing its translocation and attachment to the plasma membrane which leads to expansion of the membrane

Question/Hypothesis/Specific Aims

Specific Aims

1) Determine if DHHC8 is necessary for palmitoylation and translocation of Paralemmin-1
2) Determine if paralemmin-1 remains palmitoylated following activity
3) Determine site of DHHC8 palmitoylation of Paralemmin-1
4) Determine if synaptic activity causes DHHC8 to migrate to a location that is optimal for palmitoylating Paralemmin-1

Research Plan

Aim 1:

Observe the following before and after cLTP

- Membrane localization (GFP-tagged Paralemmin-1)
- Morphology
  - Number of filopodia and spines
  - Spine head width

Conditions:

- Knockdown of DHHC8
- Overexpression of DHHC8
- Knockdown + DHHC8 rescue
- Knockdown + DHHS8 rescue

Research Plan

Aim 2

- ABE assay: change in palmitoylated Paralemmin-1 levels

Aim 3 & 4

- Examine DHHC8-RFP and Paralemmin-1-GFP with confocal microscopy
  - Determine colocalization location
  - Determine location of DHHC8-RFP before and after cLTP
  - Antibody staining control

Expected Outcomes

- Knockdown of DHHC8, knockdown + DHHS8 rescue
  - Even after cLTP, little to no membrane localization
  - Decreased spine head width and filopodia/spines
- Overexpression of DHHC8
  - Significant increased membrane localization
  - Increased spine head width & number of filopodia/spines
- Knockdown + DHHC8 rescue
  - Control levels of membrane localization
- Palmitoylation of paralemmin-1 is constant after cLTP
- DHHC8 migrates to co-localize Paralemmin-1 after cLTP
- Increased levels of DHHC8/Paralemmin-1 co-localization after cLTP

Significance

Clinical Implications

- Understanding schizophrenia
- Investigating correlation between paralemmin-1 and tumour progression

Further Directions for Research

- Golgi Outposts
- Enzymatic Activity
- Movement Pathway, recycling endosomes

References

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