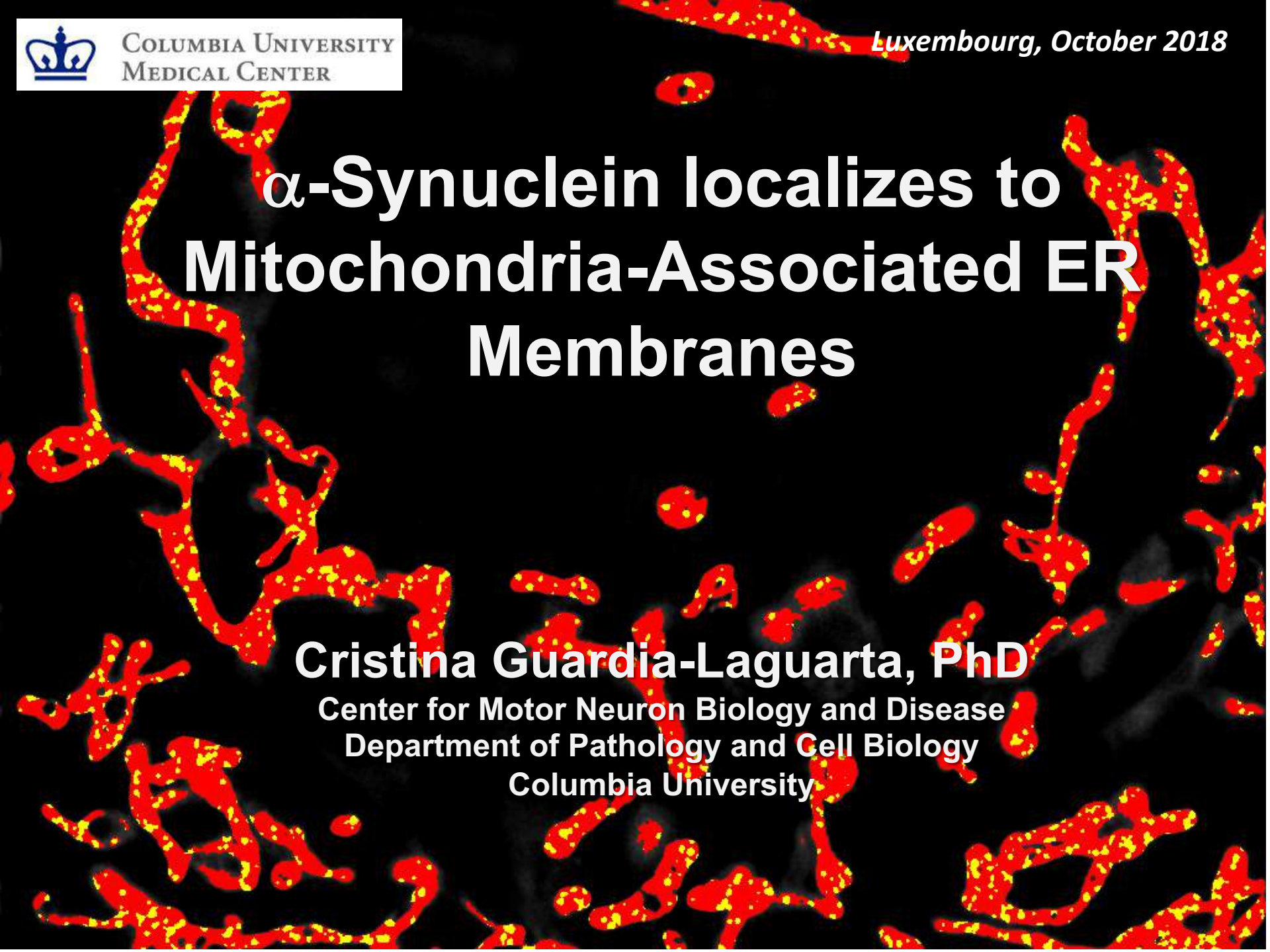

Columbia University Medical Center

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α-Synuclein localizes to Mitochondria-Associated ER Membranes

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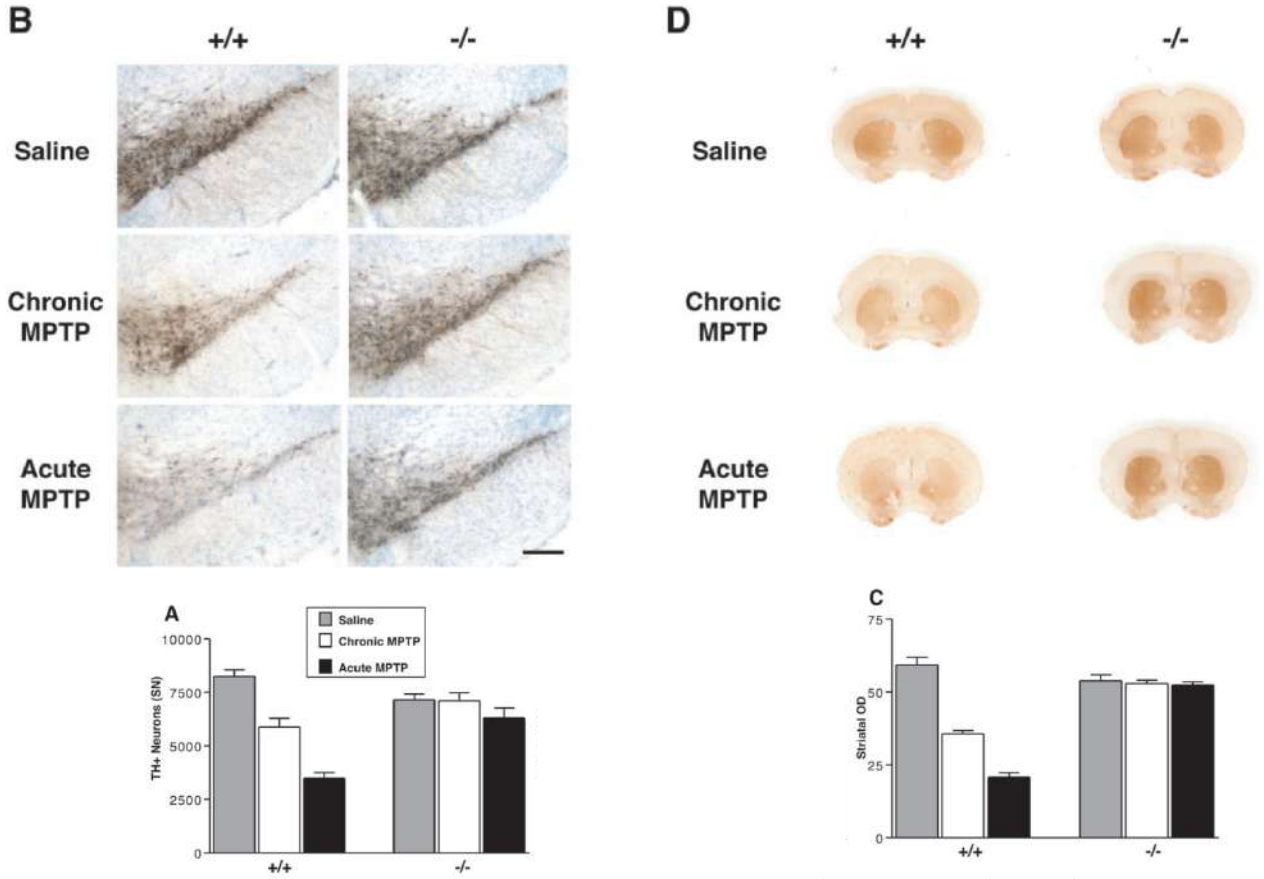
Columbia University

Why α-synuclein ?

- Its mutation or overexpression causes PD
- Lack of α-syn prevents MPTP toxicity (Dauer et al., PNAS 2002)
- The molecular mechanism of α-syn toxicity remains unknown

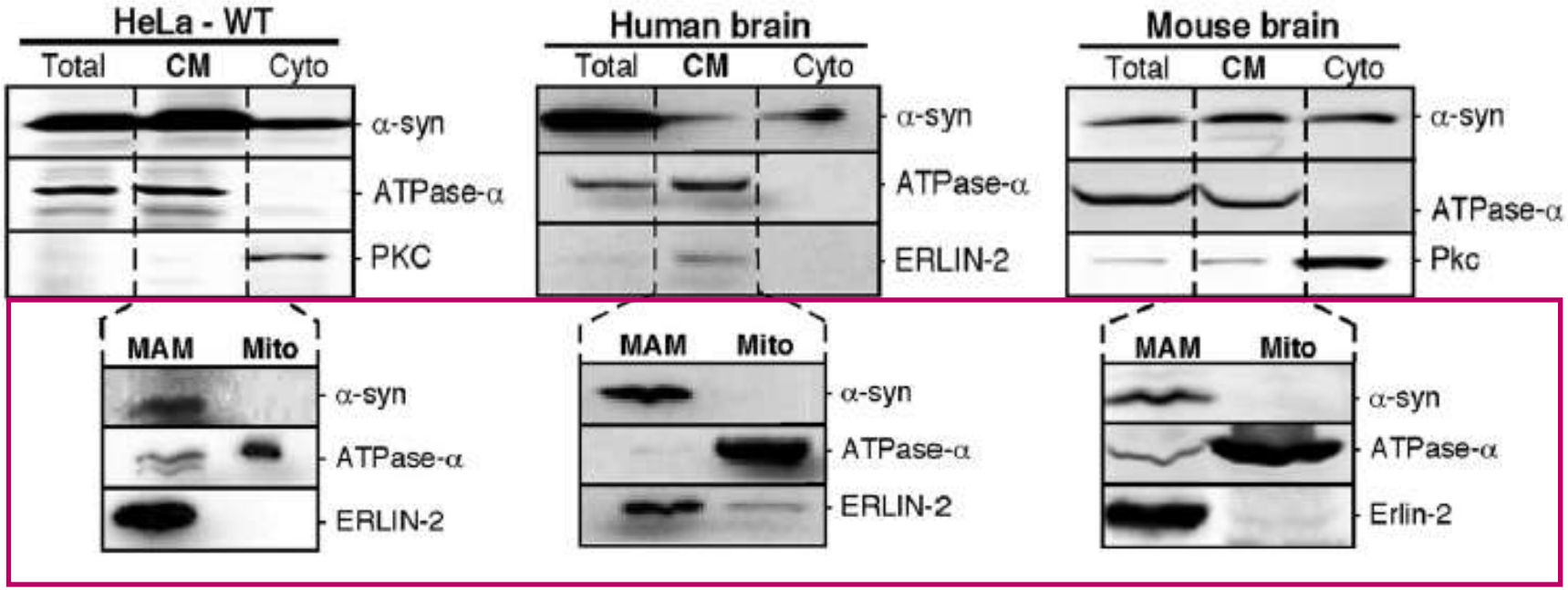


Dauer et al., Proc Natl Acad Sci USA, 99: 14524-14529, 2002.

Is α-synuclein associated with mitochondria?

- α-Syn *at* or *in* mitochondria (Li et al., 2007; Cole et al., 2008; Devi et al., 2008; Parihar et al., 2008, Shavali et al., 2008)
- Consistent with affinity for lipid bilayer membranes, especially in *lipid raft-like domain* (i.e. enriched in cholesterol and phospholipids)
- None of the prediction algorithms (e.g. MitoProt) recognize α-syn as a mitochondrially targeted protein
- Two tests*:*
  - *In vitro* import assay of [^{35}S-Met/Cys]-labeled WT and mutant α-syn
  - Western blots of subcellular fractionations

Where in the cell is α-synuclein located?



Percoll gradient centrifugation

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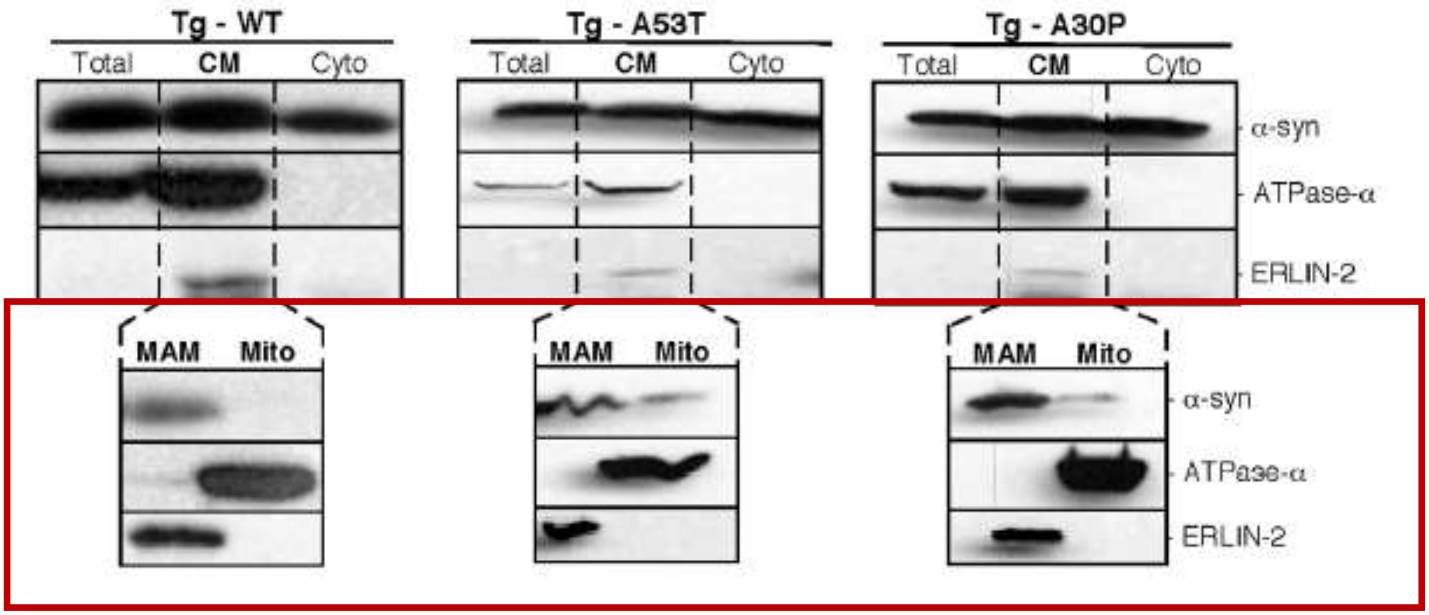
But mutant α-synuclein…



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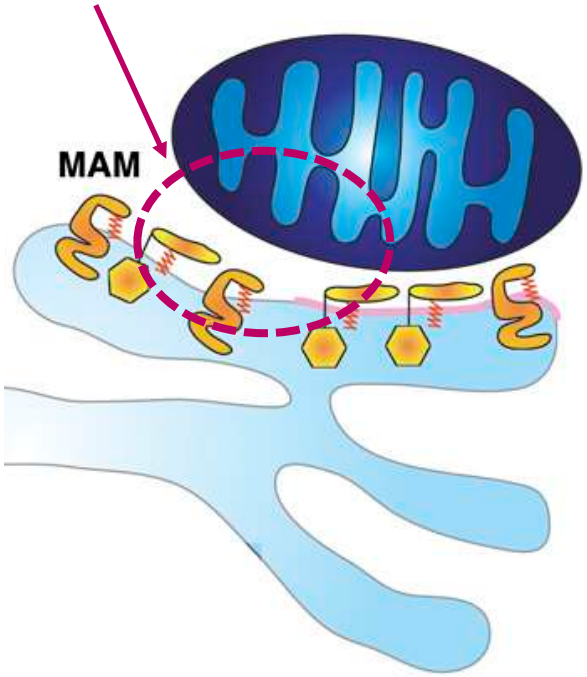
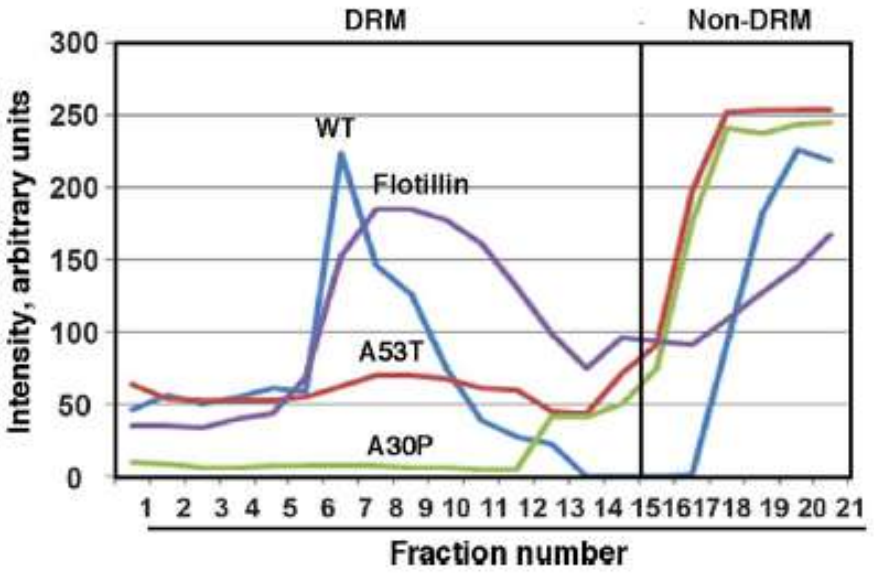
And WT α-Syn is recovered from raft-like domains…

Detergent-resistant membranes (DRMs) from crude mitochondria



Sucrose gradient centrifugation



Flotilin = Raft marker

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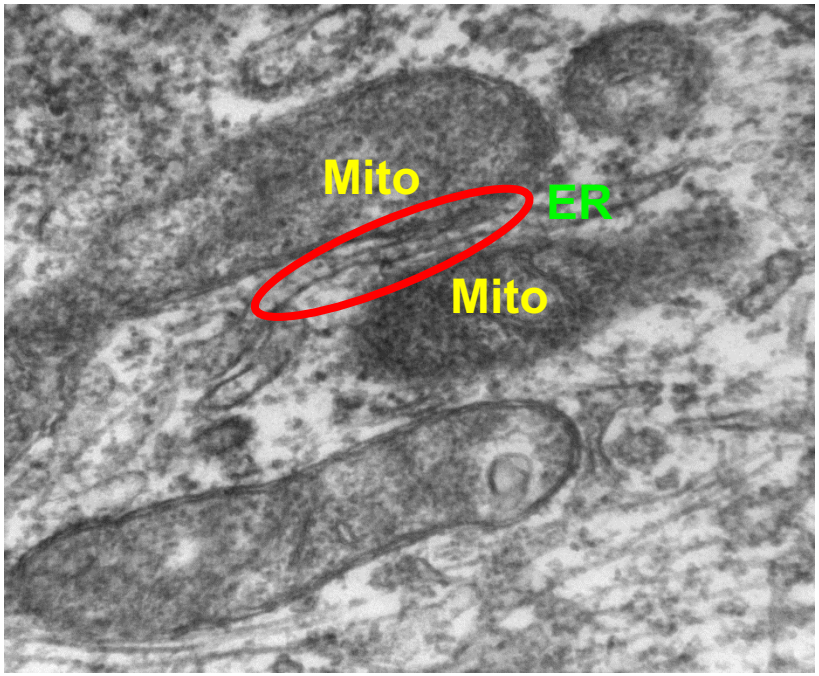
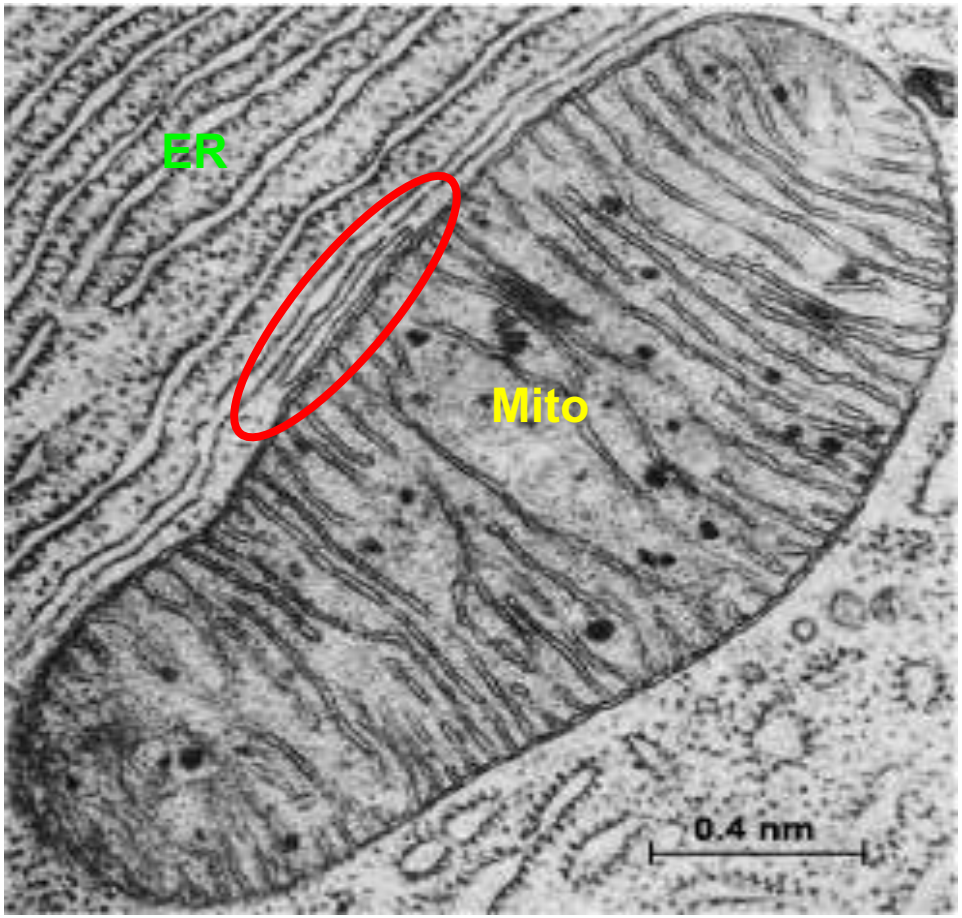
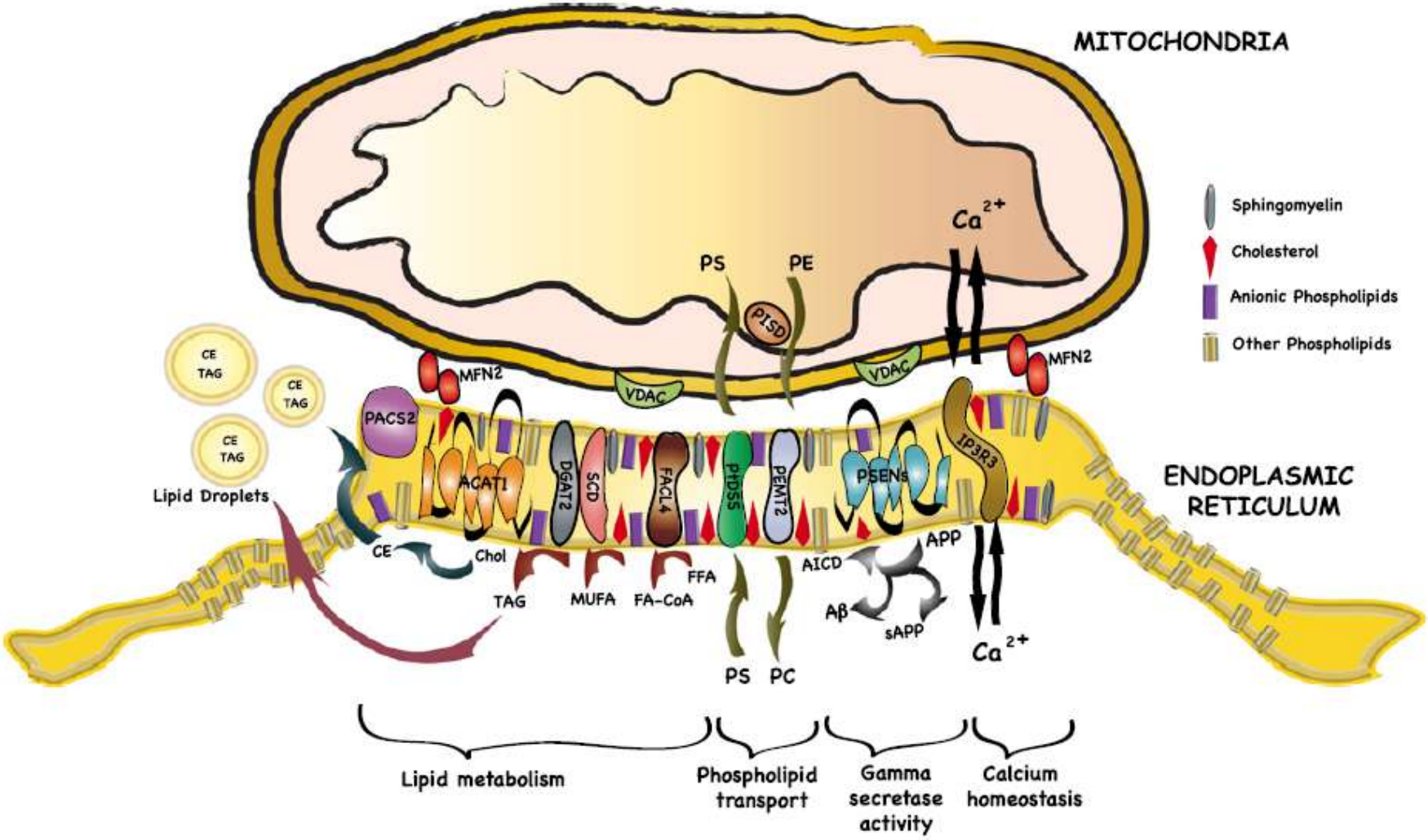
Mitochondria-Associated Membranes (MAM)



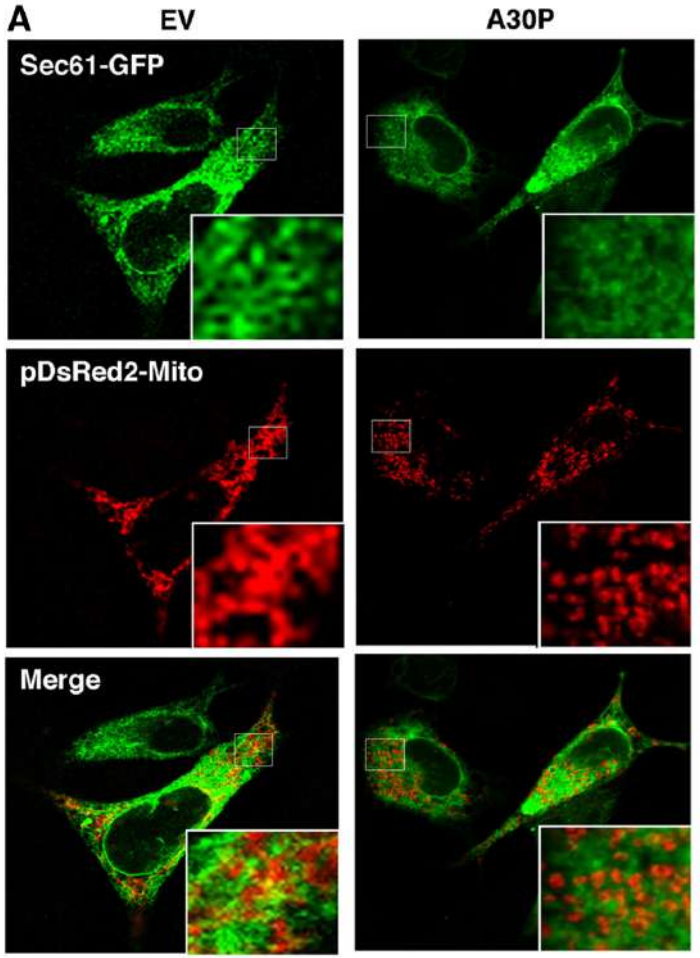


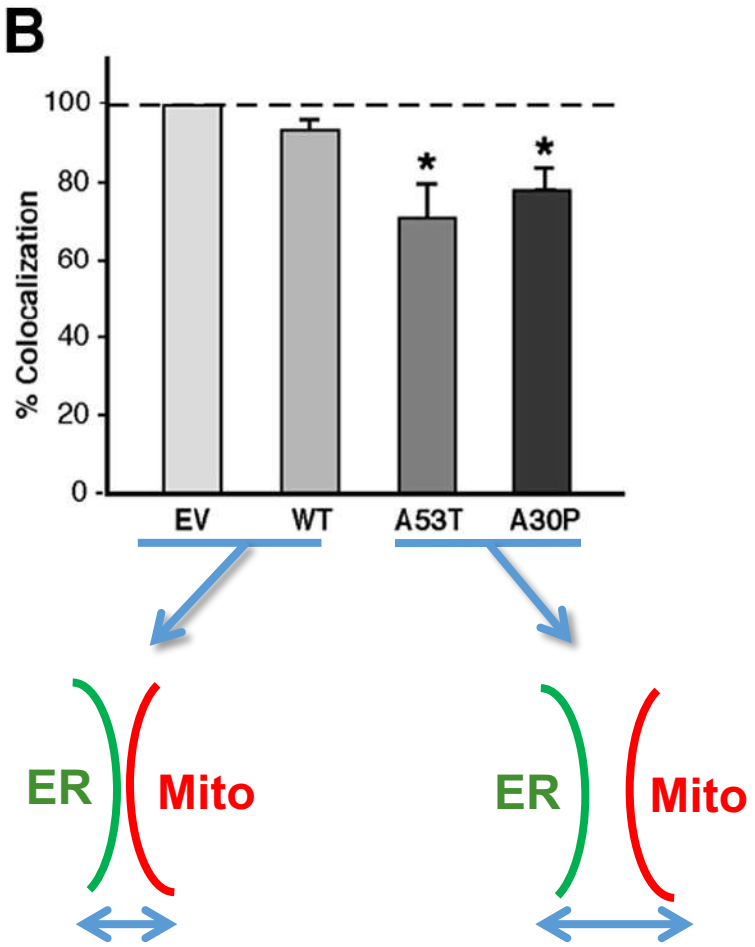
Estela Area


MITOCHONDRIA
Ca 2+
PS
PE
PISD
MFN2
VDAC
VDAC
MFN2
Sphingomyelin
Cholesterol
Anionic Phospholipids
Other Phospholipids
CE TAG
CE TAG
CE TAG
Lipid Droplets
PACS2
ACAT1
DGAT2
SCD
FACL4
ptDS5
PEMT2
PSENs
IP3R3
ENDOPLASMIC RETICULUM
CE
Chol
TAG
MUFA
FA-CoA
FFA
PS
PC
AICD
APP
Aβ
sAPP
Ca 2+
Lipid metabolism
Phospholipid transport
Gamma secretase activity
Calcium homeostasis

Estela Area

Mutations in α-synuclein reduce ER-mitochondria apposition





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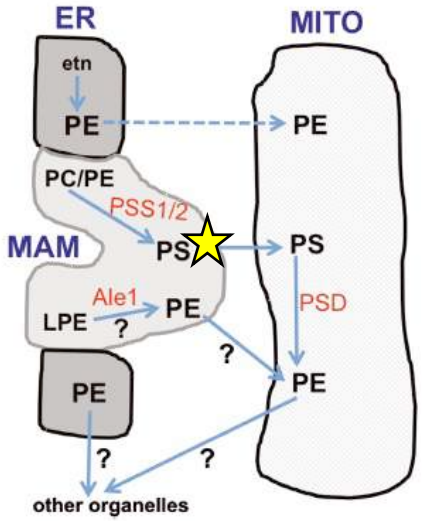
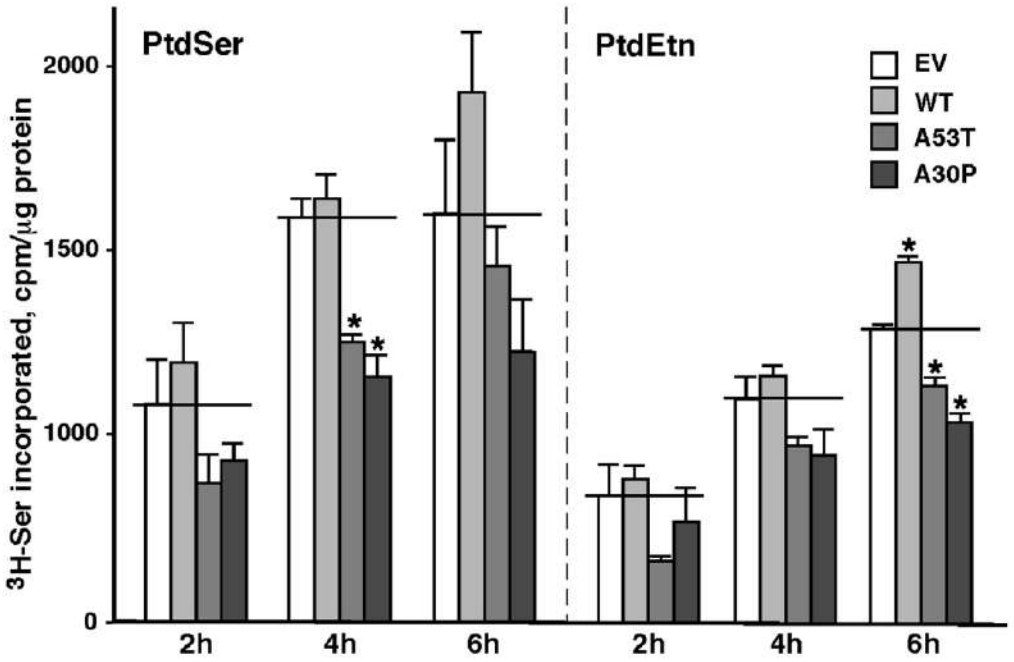
α-Syn mutants alter phospholipid transfer





A53T and A30P mutations caused a significant decrease in PtdSer and PtdEtn synthesis, inducing a decrease in MAM activity

Guardia-Laguarta C et al., J Neurosci 2014

Take home messages

- WT α-syn, apart from being cytosolic, is also in MAM
- Mutant α-syn altered distribution between cytosol and MAM
- This misallocation is associated with:
  - ↓ ER–mitochondria apposition
  - ↓ Lipid biosynthesis
- α-Syn at the ER–mitochondrial may explain previous reports on α-syn association with *mitochondria* (using CM preparations) and *nerve terminal synaptic vesicles*
- α-syn in MAM may help us understand the role α-syn plays in PD pathogenesis

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