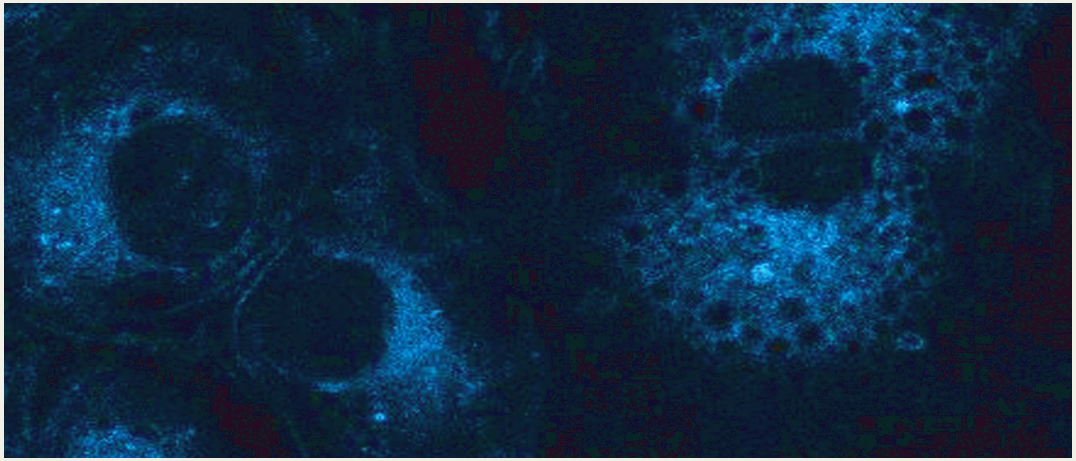
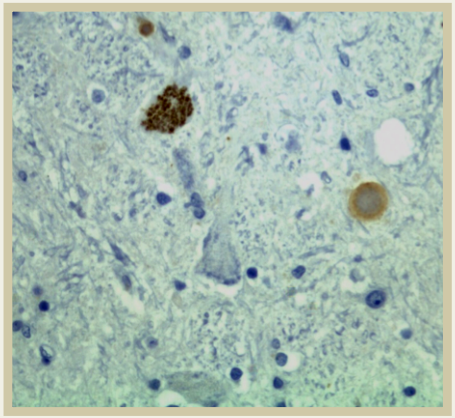
Restoration of Rab1 levels mitigates endoplasmic reticulum stress in hippocampal cells during protein aggregation

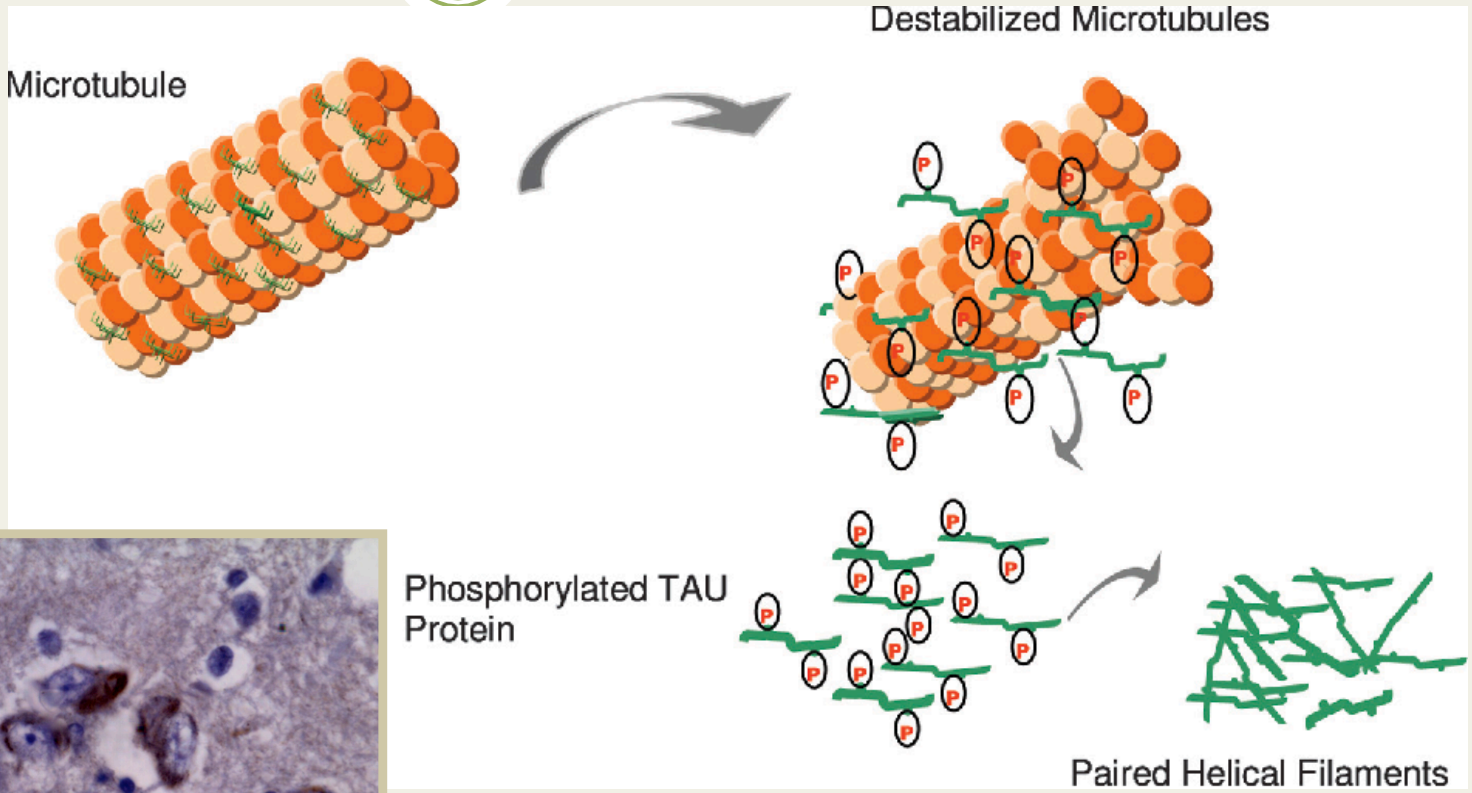


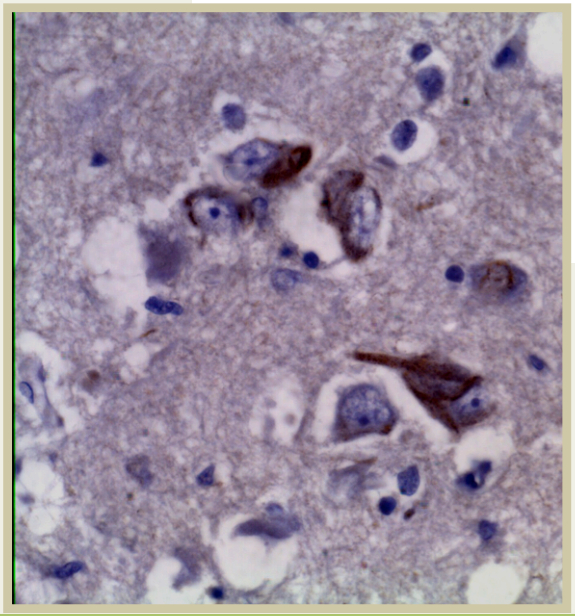
MERARI F.R. FERRARI
DEPT. OF GENETICS AND EVOLUTIONARY BIOLOGY
INSTITUTE OF BIOSCIENCES –USP
merari@usp.br

Presence of protein aggregates in the course of neurodegeneration



alfa-synuclein
(substantia nigra)





hyperphosphorylated Tau
(hippocampus)

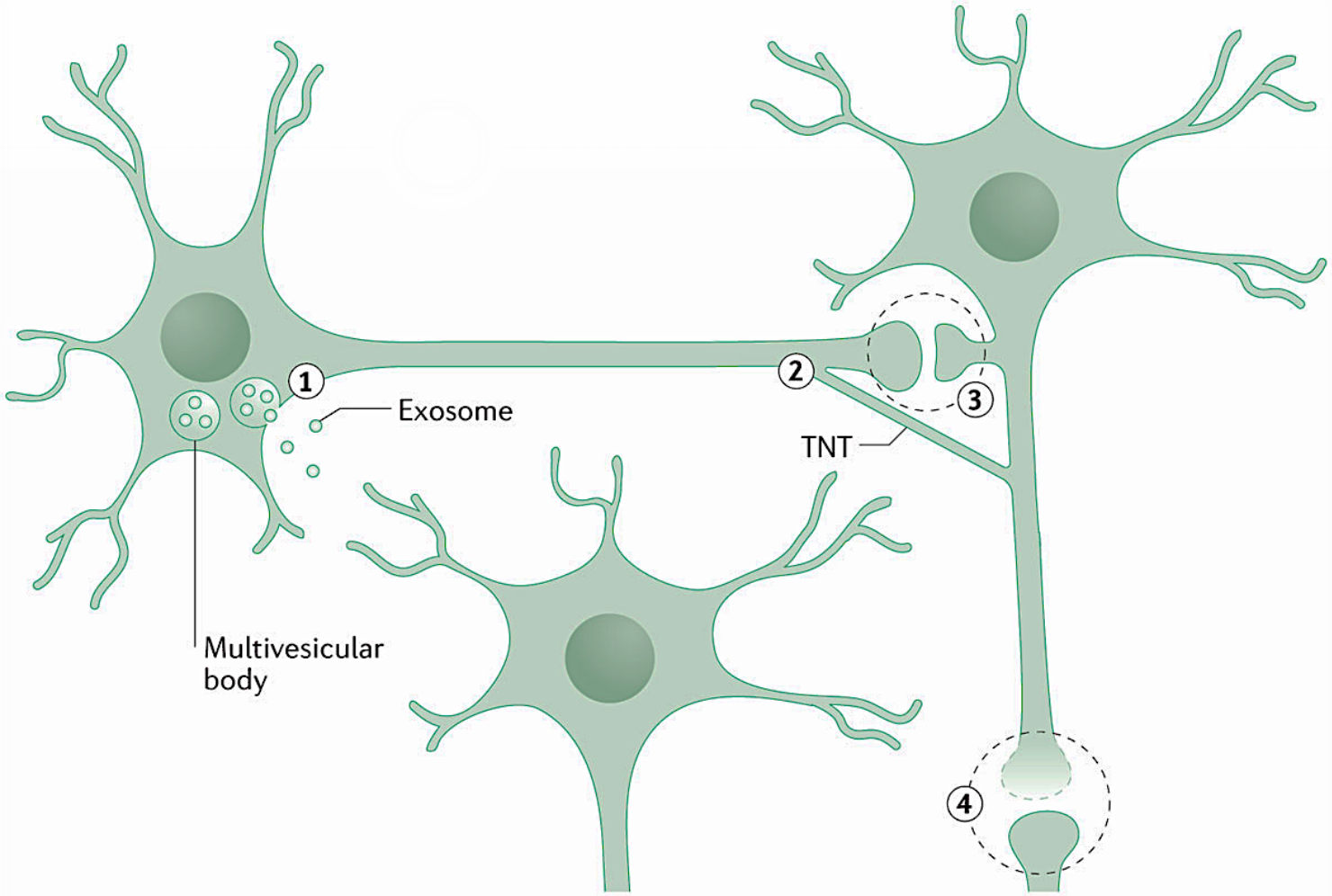
Dementia & neuropsychologia 2009 DOI:10.1590/S1980-57642009DN30300003

Protein oligomers/fibrils are considered more toxic than aggregates

Spread to surrounding cells

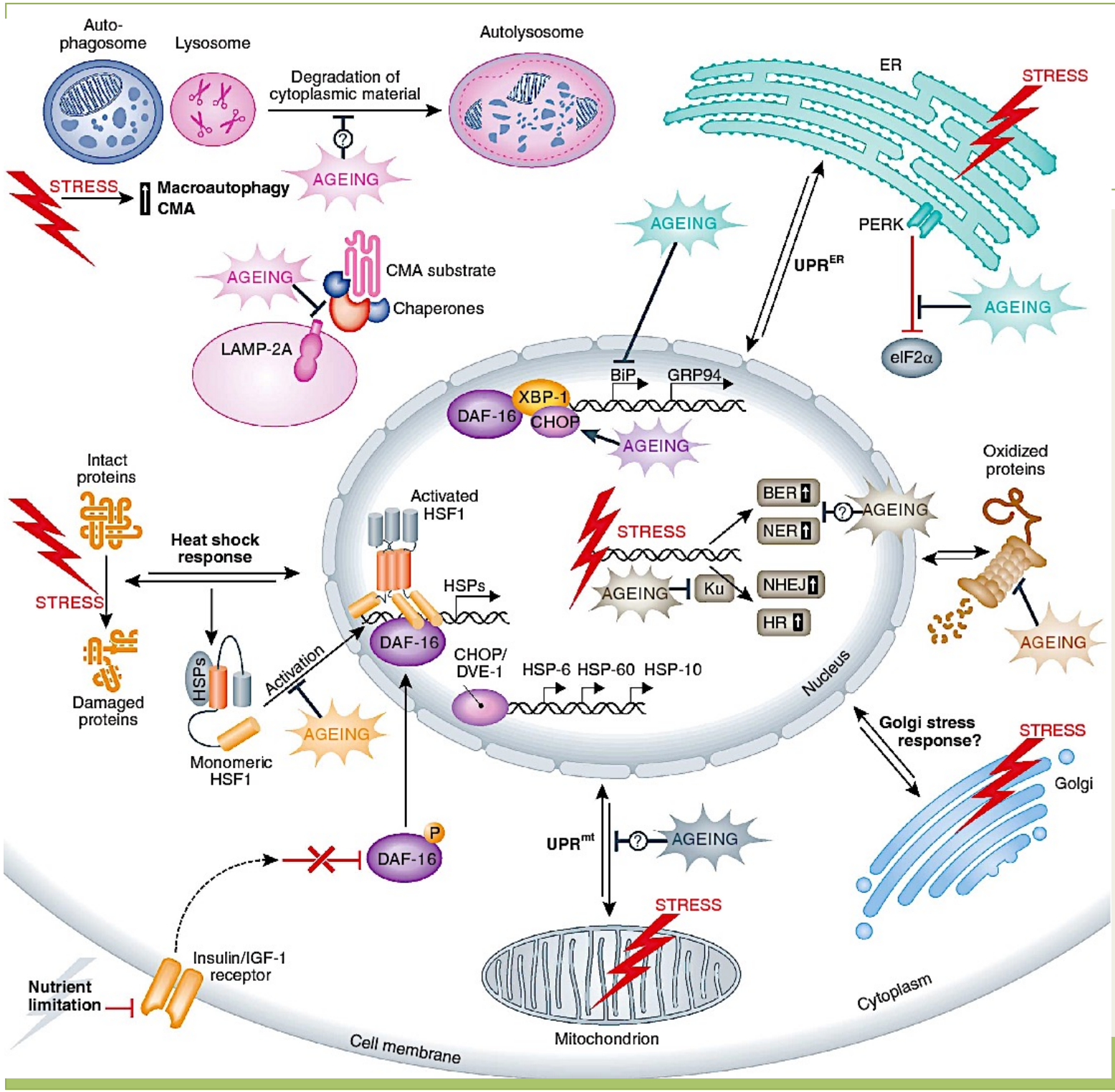
Trigger inflammation

Disseminate proteinopathy



Nature Reviews | Neuroscience

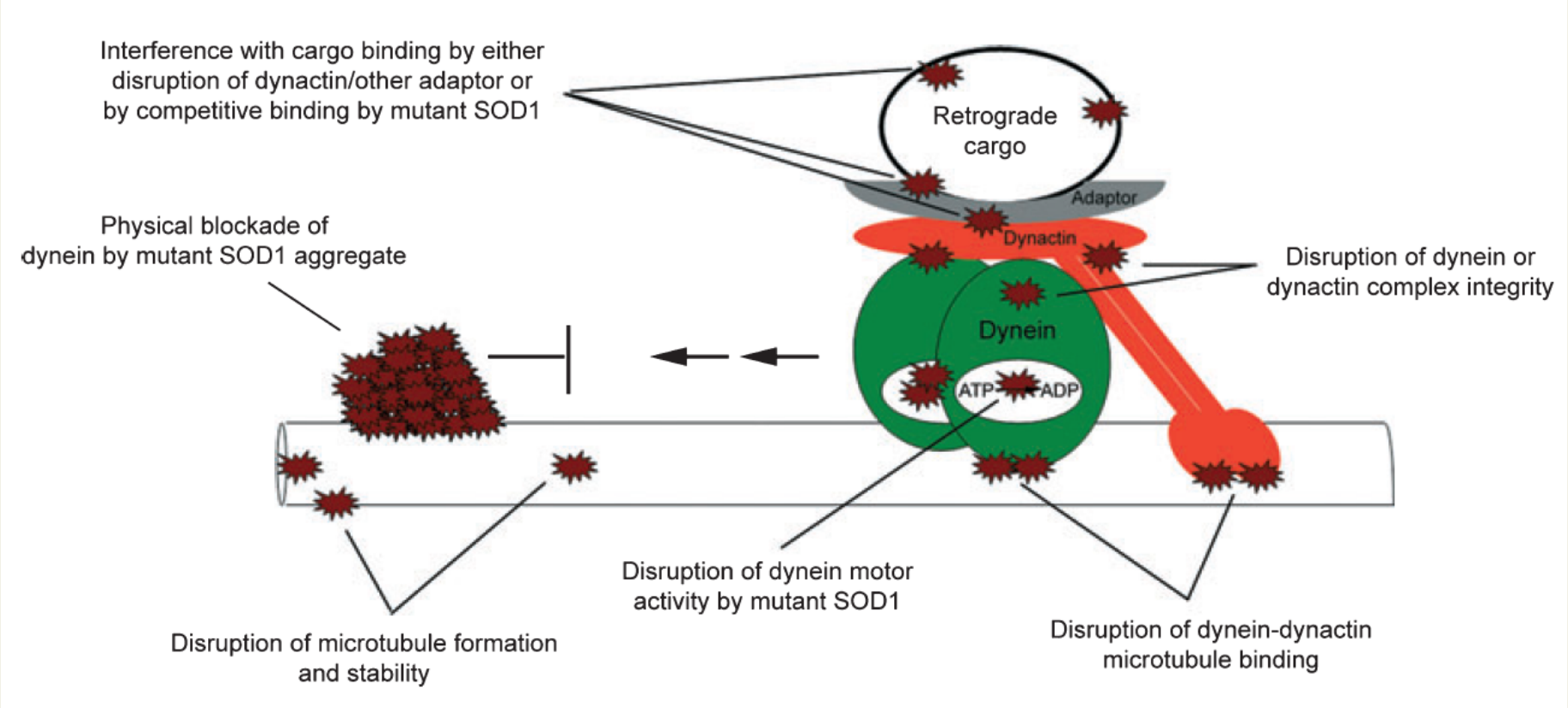
Ageing



Ageing affects many organelles/ processes

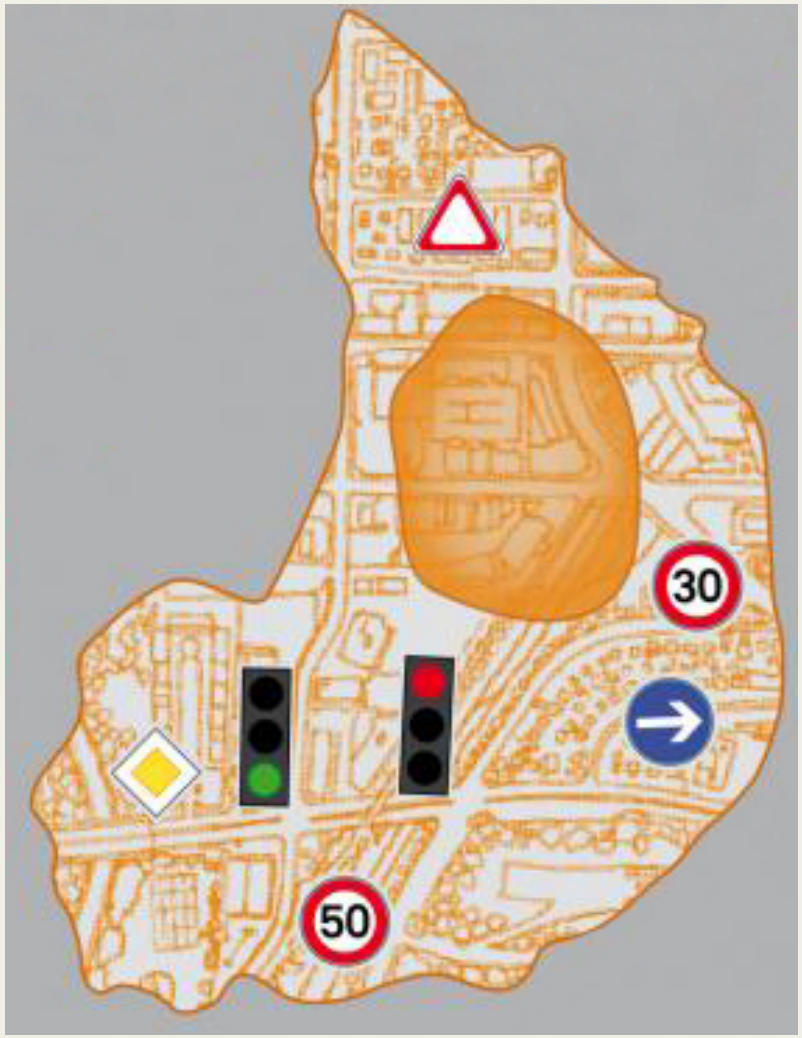
Main risk factor for neurodegeneration

Protein aggregates impair intracellular dynamics



J. Neurochem. (2008) 106: 495–505

Cause or effect?



Dysruption of intracellular traffic occurs prior to protein aggregation and might lead to accumulation of misfolded proteins

Experimental mutations in Rabs and other traffic proteins increase aggregation of misfolded proteins in model of PD

Intracellular trafficking is impaired before protein aggregation

ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research Paper Acta Neurobiol Exp 2013, 73: 541–556

Dynein c1h1, dynactin and syntaphilin expression in brain areas related to neurodegenerative diseases following exposure to rotenone

Rodrigo S. Chaves, Thaiany Q. Melo, Aline M. D'Unhao, Karen L.G. Farizatto, and Merari F.R. Ferrari*

Department of Genetics and Evolutionary Biology, Institute for Biosciences, University of Sao Paulo, Sao Paulo, SP, Brazil, *Email: merari@usp.br

Motor proteins related to retrograde and anterograde traffic is misregulated prior to protein aggregation

In vivo and in vitro models of protein aggregation

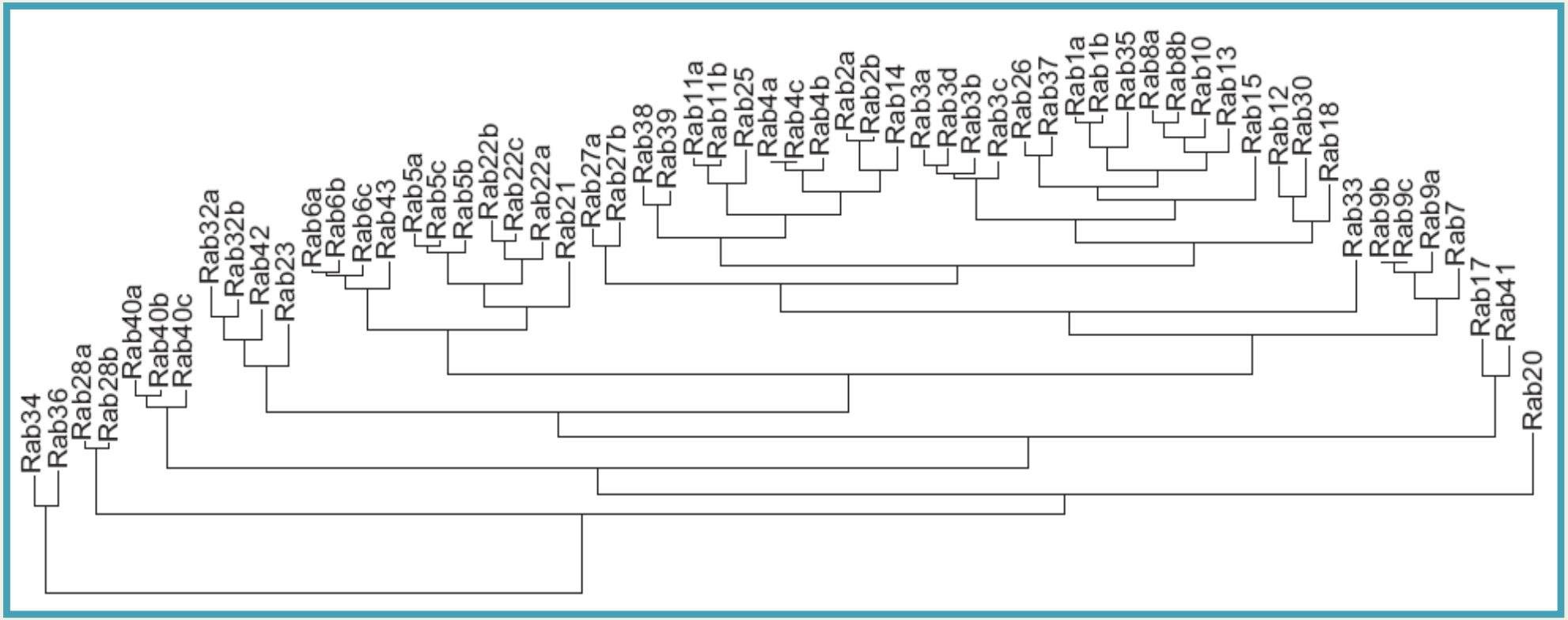
Cell Mol Neurobiol (2013) 33:327–335
DOI 10.1007/s10571-012-9898-z

ORIGINAL RESEARCH

Rotenone-Dependent Changes of Anterograde Motor Protein Expression and Mitochondrial Mobility in Brain Areas Related to Neurodegenerative Diseases

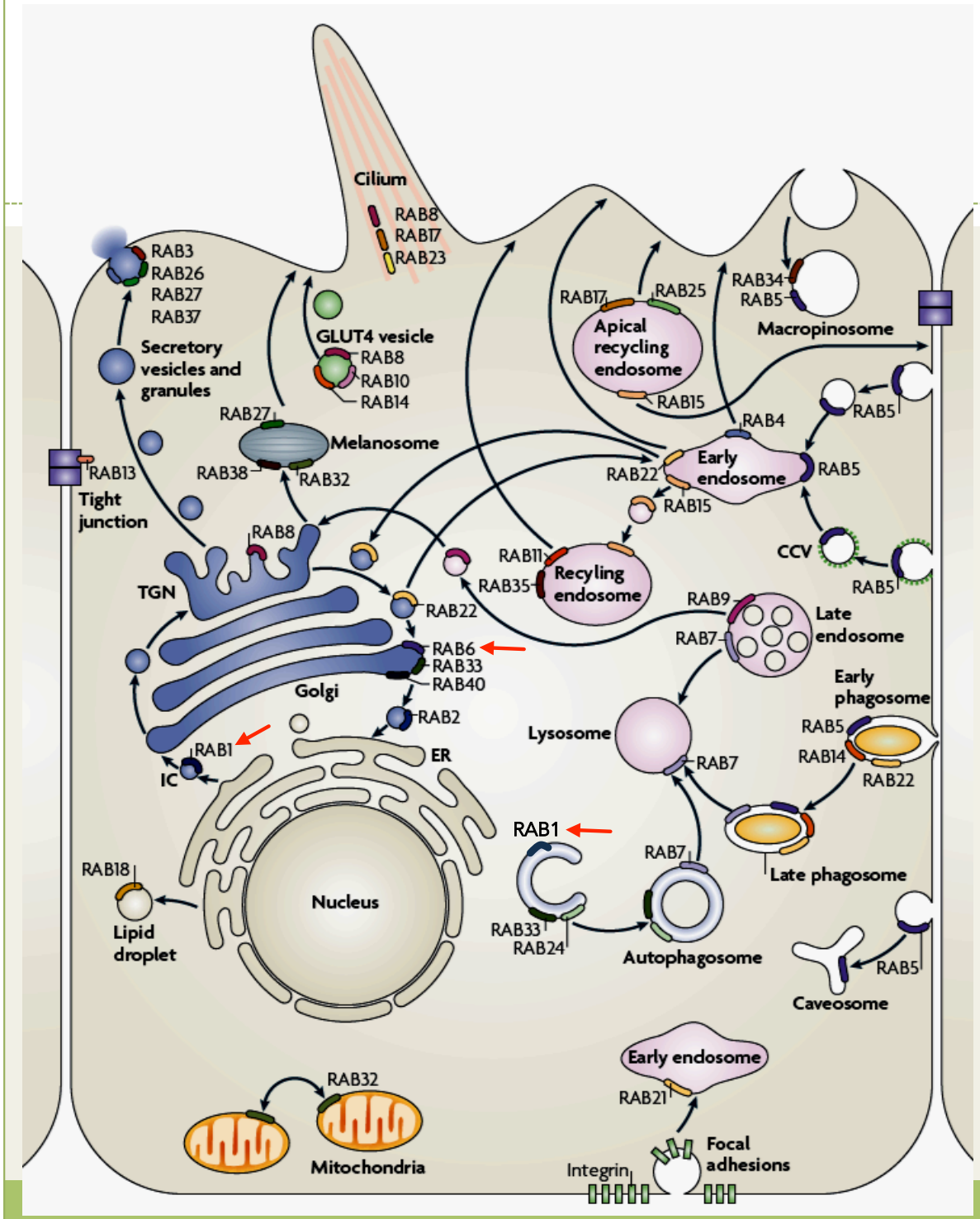
Thaiany Q. Melo · Aline M. D'unhao · Stephanie A. Martins · Karen L. G. Farizatto · Rodrigo S. Chaves · Merari F. R. Ferrari

Rab proteins: Intracellular dynamics



Over 60 Rabs were described to date

Rab Proteins

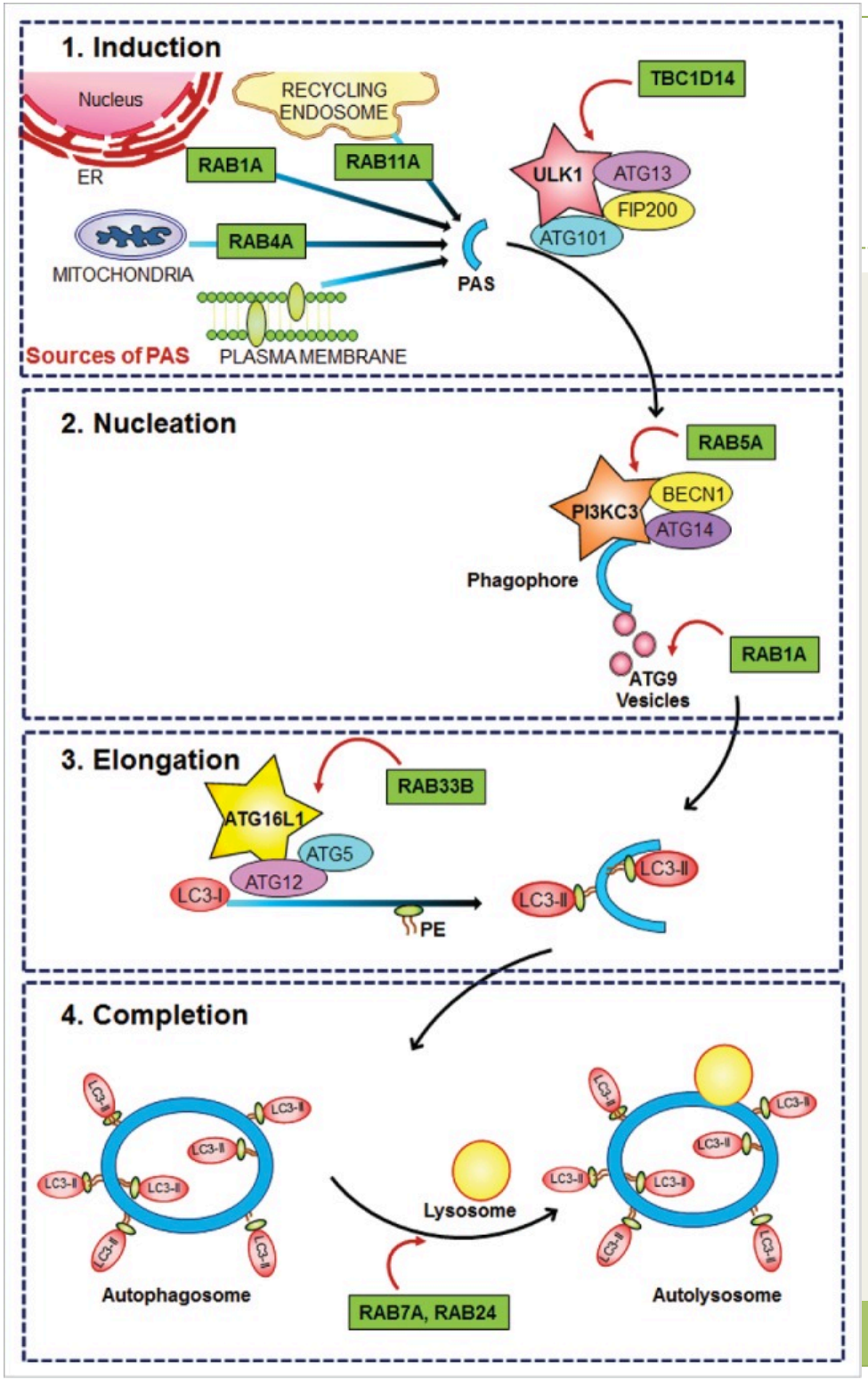


Delivery of specific cargos to specific cell domains

Responsible for vesicles budding, mobility between intracellular compartments, anchoring of organelles, transport and fusion of mitochondria, recycling and autophagy

Good drug target

Nature Rev. Mol. Cell. Biol. (2009) 10:513-525 (modified)



Rabs and autophagy

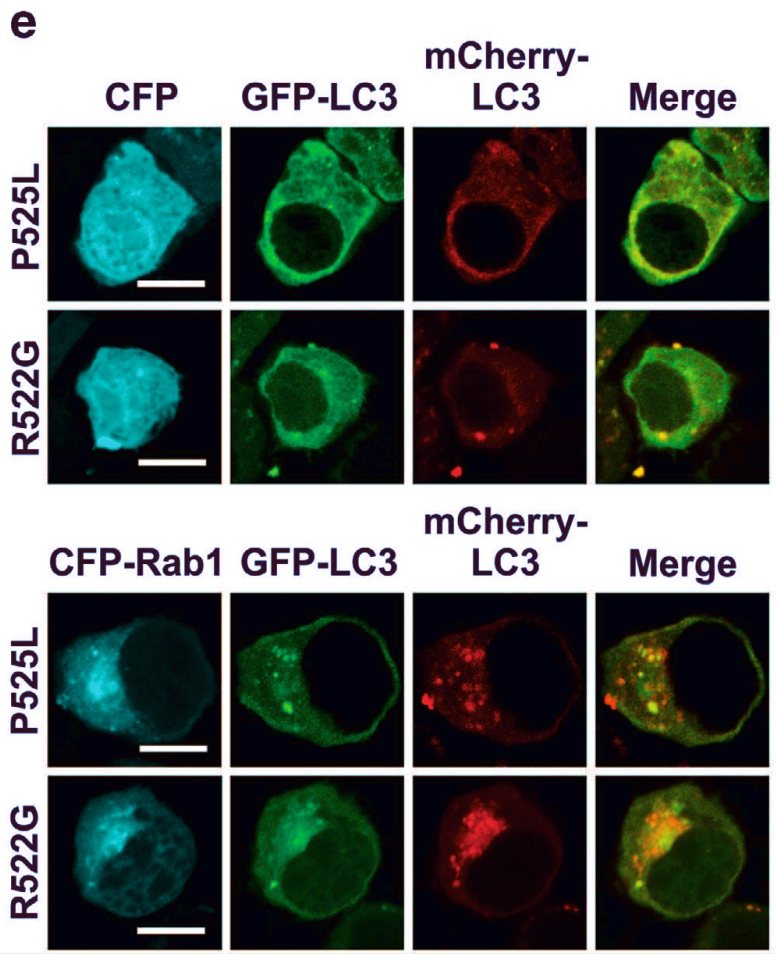
The role of Rab1 in the autophagy pathway:

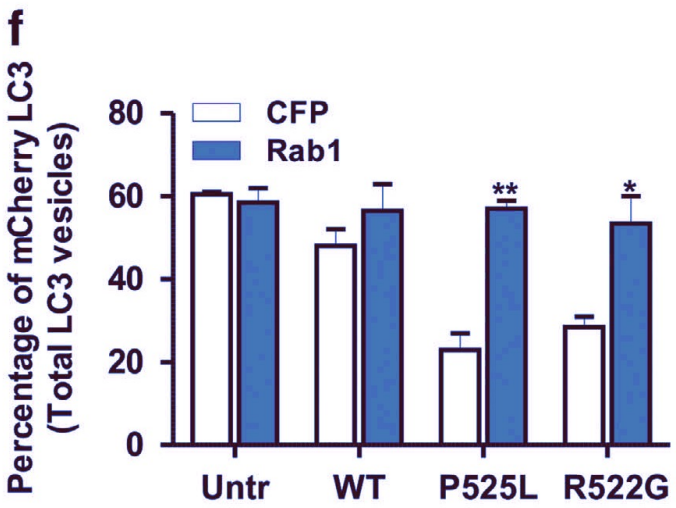
Recruitment of membranes (ER) to form autophagic vacuole

Autophagy 2016 12(5): 900–904

Rab1 in a cellular model of ALS

Rab1 overexpression restores autophagosome formation in cells expressing mFUS





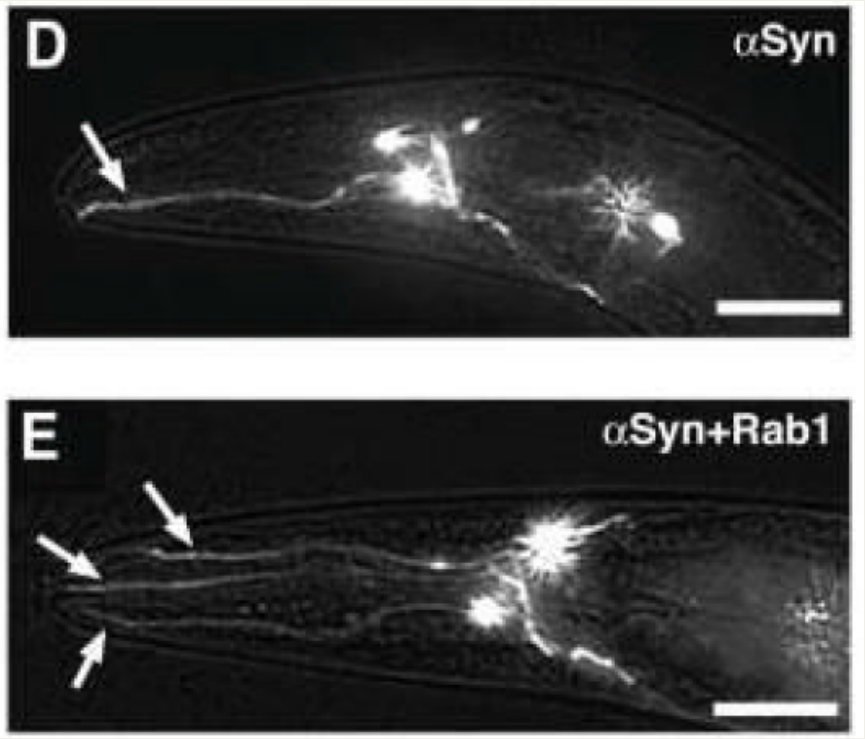
FUS mutations have been described in human sporadic and familial ALS

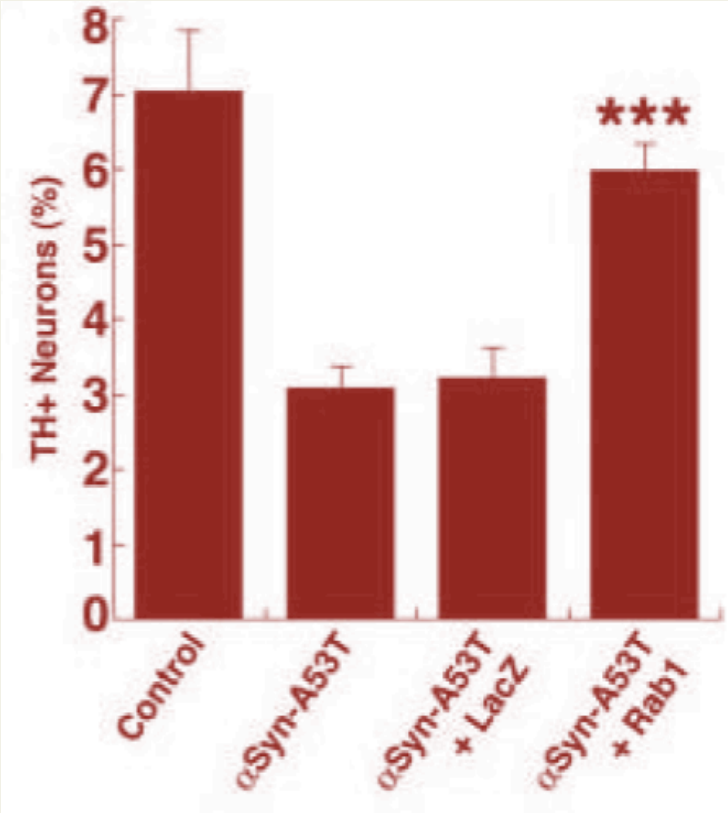
FUS-positive inclusions are present in affected tissues.

FUS roles are implicated in RNA processing and transport.

Cell Death Discovery (2015) 1, 15030; doi:10.1038/cddiscovery.2015.30

Rab1 expression in an animal model of PD





Rab1 overexpression prevents dopaminergic neurons death

Role of restoration of ER-Golgi trafficking and formation of autophagosomes vesicles.

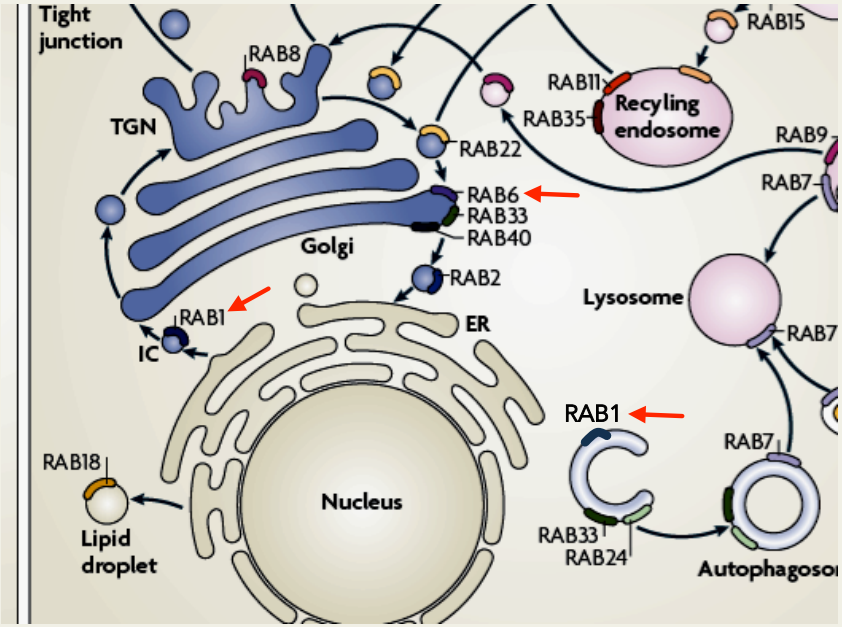
Science (2006) 313(5785): 324–328.

Aim 1

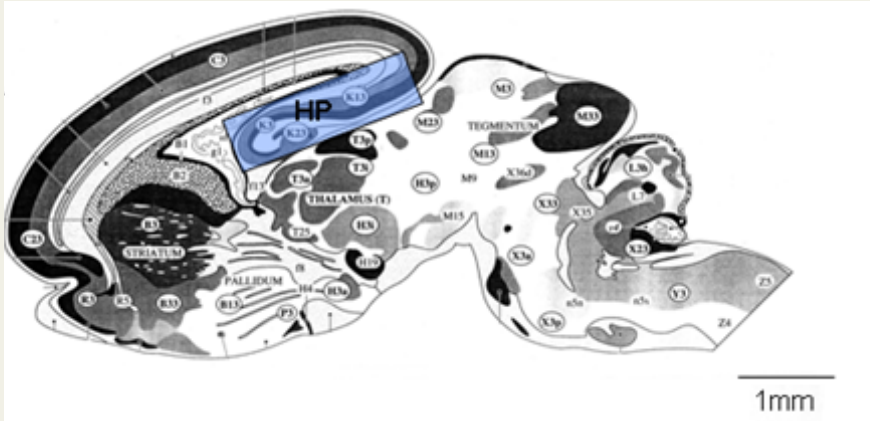
- To evaluate the expression of Rab1 and Rab6 in animal and cellular models of protein aggregation

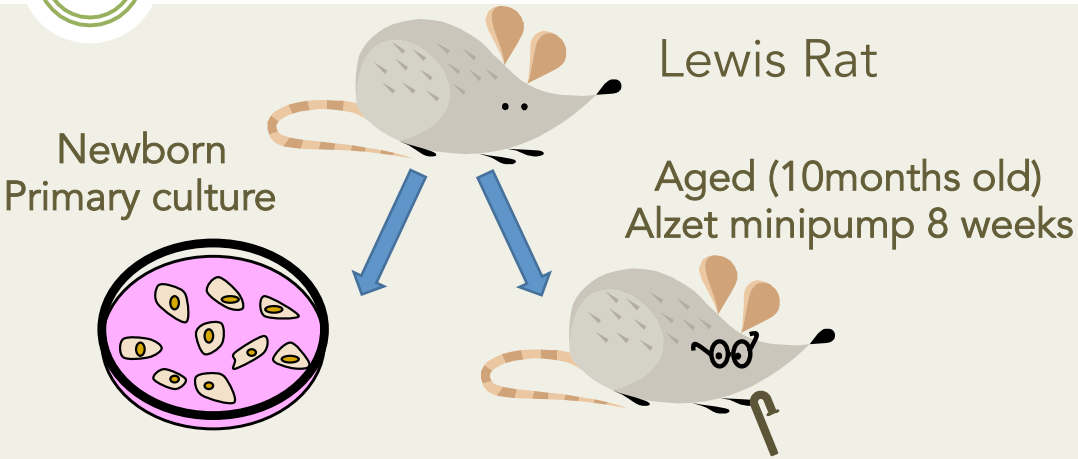
Nathan R. Lima

Dr. Thaiany Q. Melo



Methods





- ✓ Inhibition of mitochondrial complex I
- ✓ Action upon neurons and glial cells
- ✓ Promotes protein aggregation
- ✓ Increases oxidative stress
- ✓ Mimics ageing

Chaves *et al. BMC Neuroscience* 2010, **11**:144
http://www.biomedcentral.com/1471-2202/11/144

BMC Neuroscience

RESEARCH ARTICLE Open Access

Protein aggregation containing beta-amyloid, alpha-synuclein and hyperphosphorylated tau in cultured cells of hippocampus, substantia nigra and locus coeruleus after rotenone exposure

Rodrigo S Chaves, Thaiany Q Melo, Stephanie A Martins, Merari FR Ferrari*

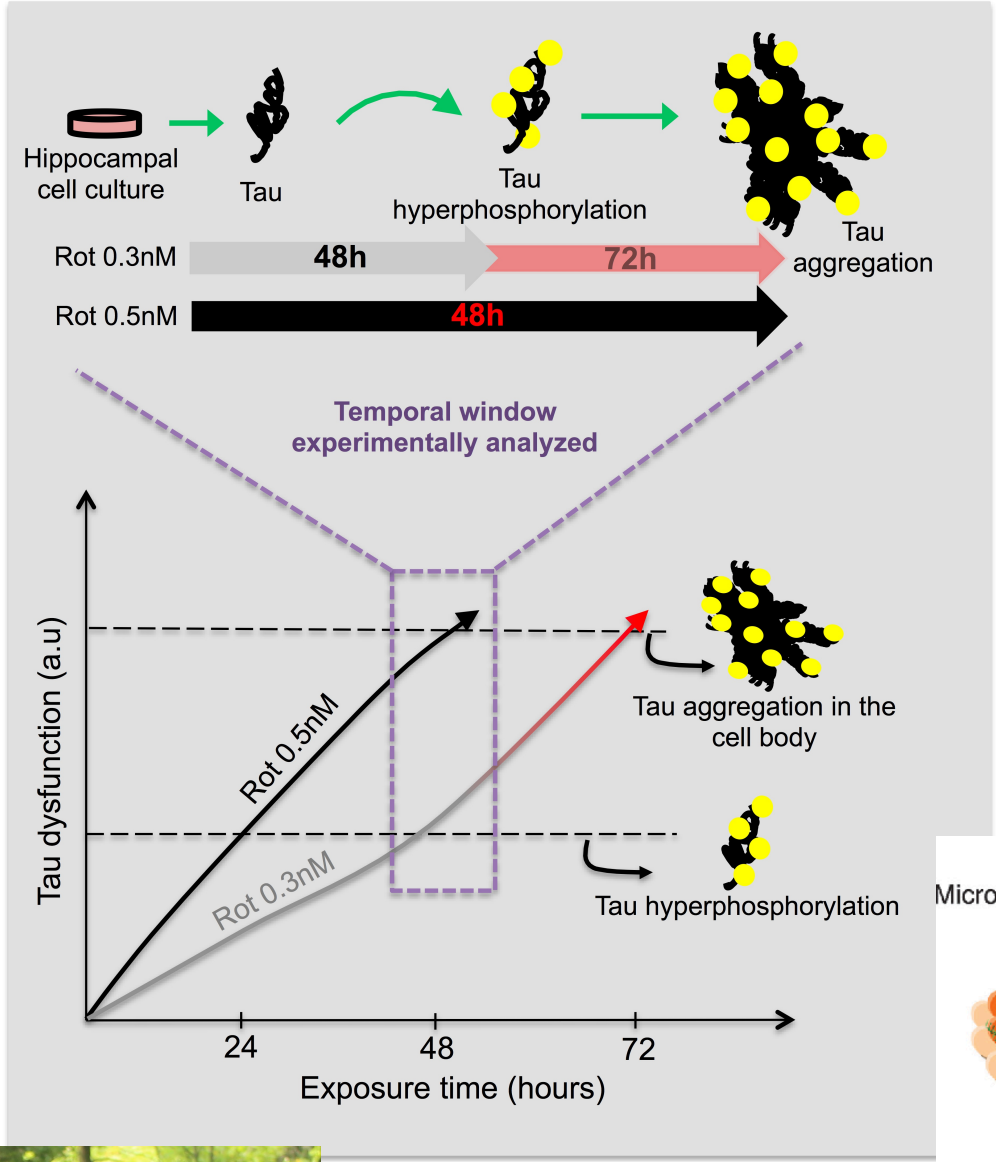
Evaluation Studies > Arq Neuropsiquiatr, 74 (9), 737-744 Sep 2016

Aged Lewis Rats Exposed to Low and Moderate Doses of Rotenone Are a Good Model for Studying the Process of Protein Aggregation and Its Effects Upon Central Nervous System Cell Physiology

Michael F Almeida, Carolliny M Silva ... Merari F R Ferrari + expand

PMID: 27706423 DOI: 10.1590/0004-282X20160121

In vitro model of protein aggregation



Rotenone 0.3nM:

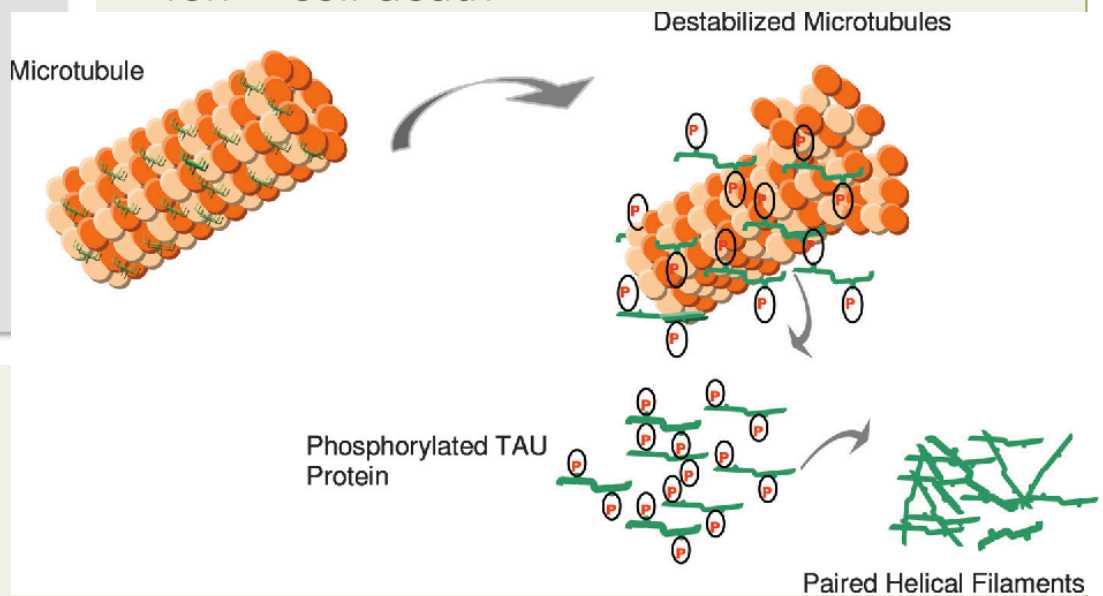
48h = prior protein aggregation (increased pTau levels)

72h = protein aggregation

Rotenone 0.5nM:

48h = protein aggregation

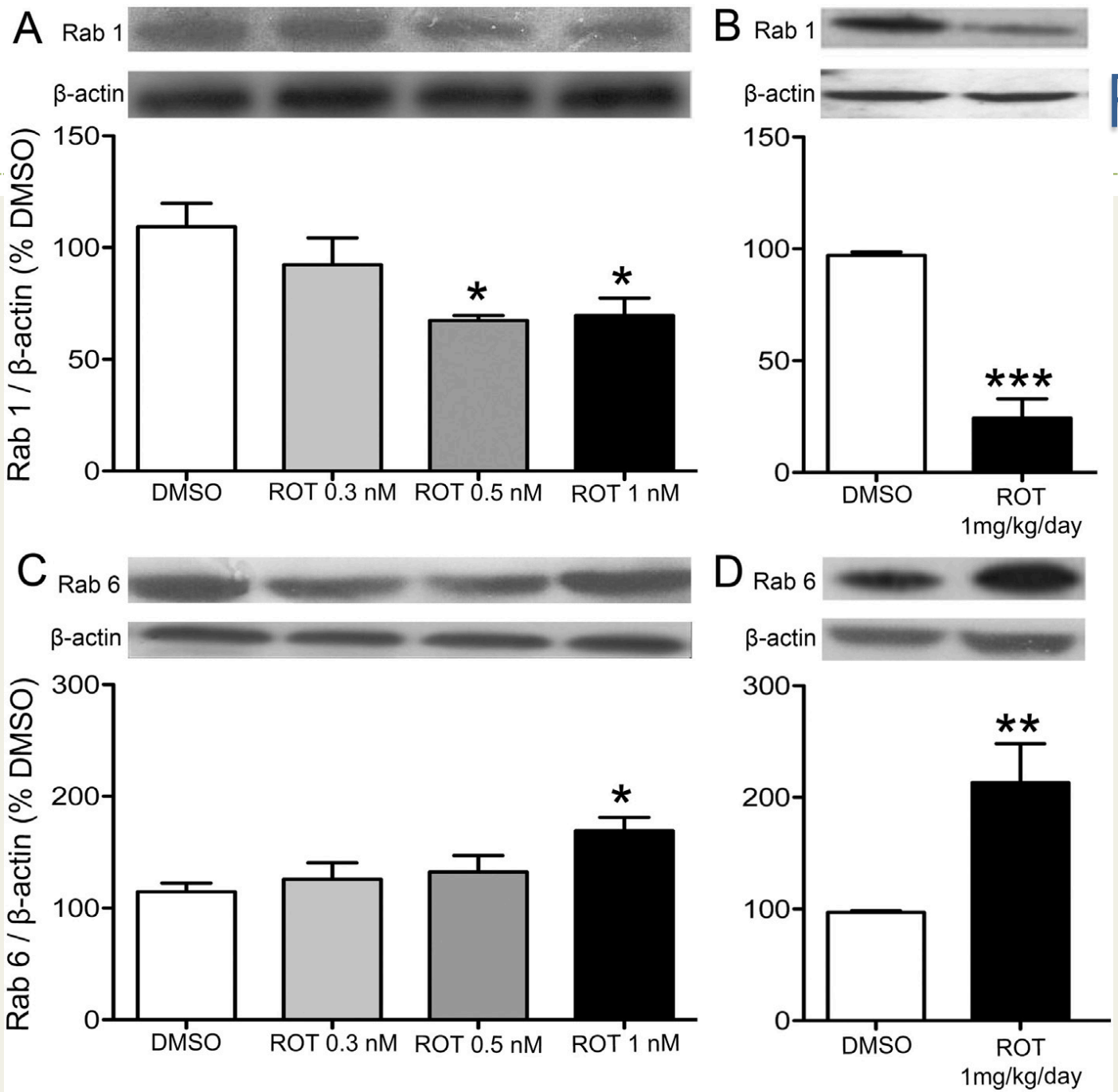
Rotenone 1nM:
48h = cell death



Dr. Rodrigo S. Chaves

Dementia & neuropsychologia 2009 DOI:10.1590/S1980-57642009DN30300003
Chaves et al. Ferrari (2016) http://dx.doi.org/10.1016/j.ibror.2016.09.001

Results – Aim 1



48h exposure of rotenone (cultures)

Decreased Rab1 levels in the presence of protein oligomers and aggregates

- decreased ER-Golgi trafficking, decreased autophagosome formation

Increased Rab6 levels after protein aggregation

- increased Golgi-ER

Student's T-test or one-way ANOVA
*p<0.05, **p<0.01, ***p<0.001 compared to DMSO. N=5

Lima et al. Ferrari Neuroscience (2019) 419:5-13

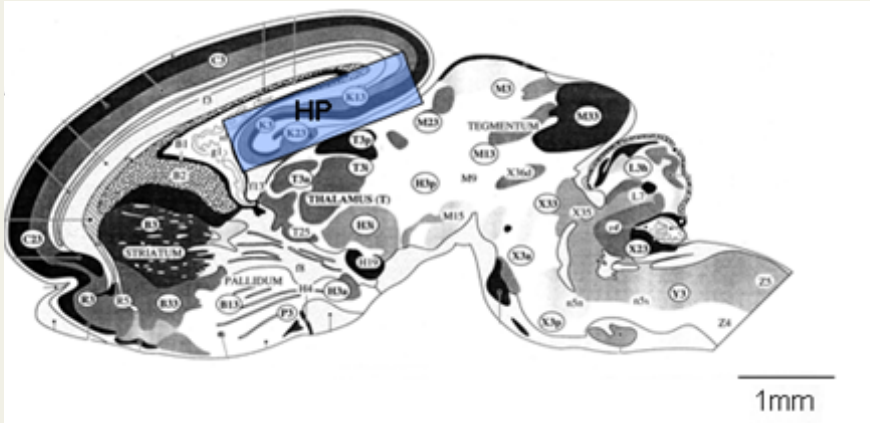
Aims 2 and 3

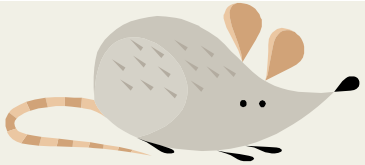
2- How is ER physiology affected by protein aggregation?

3- What if Rab1 levels were restored?

Nathan R. Lima

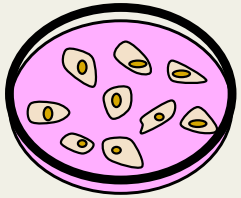
Methods



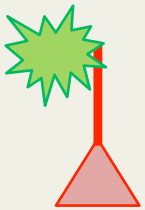


Lewis Rat

Newborn
Primary culture

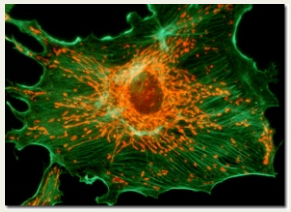


Immunocitochemistry



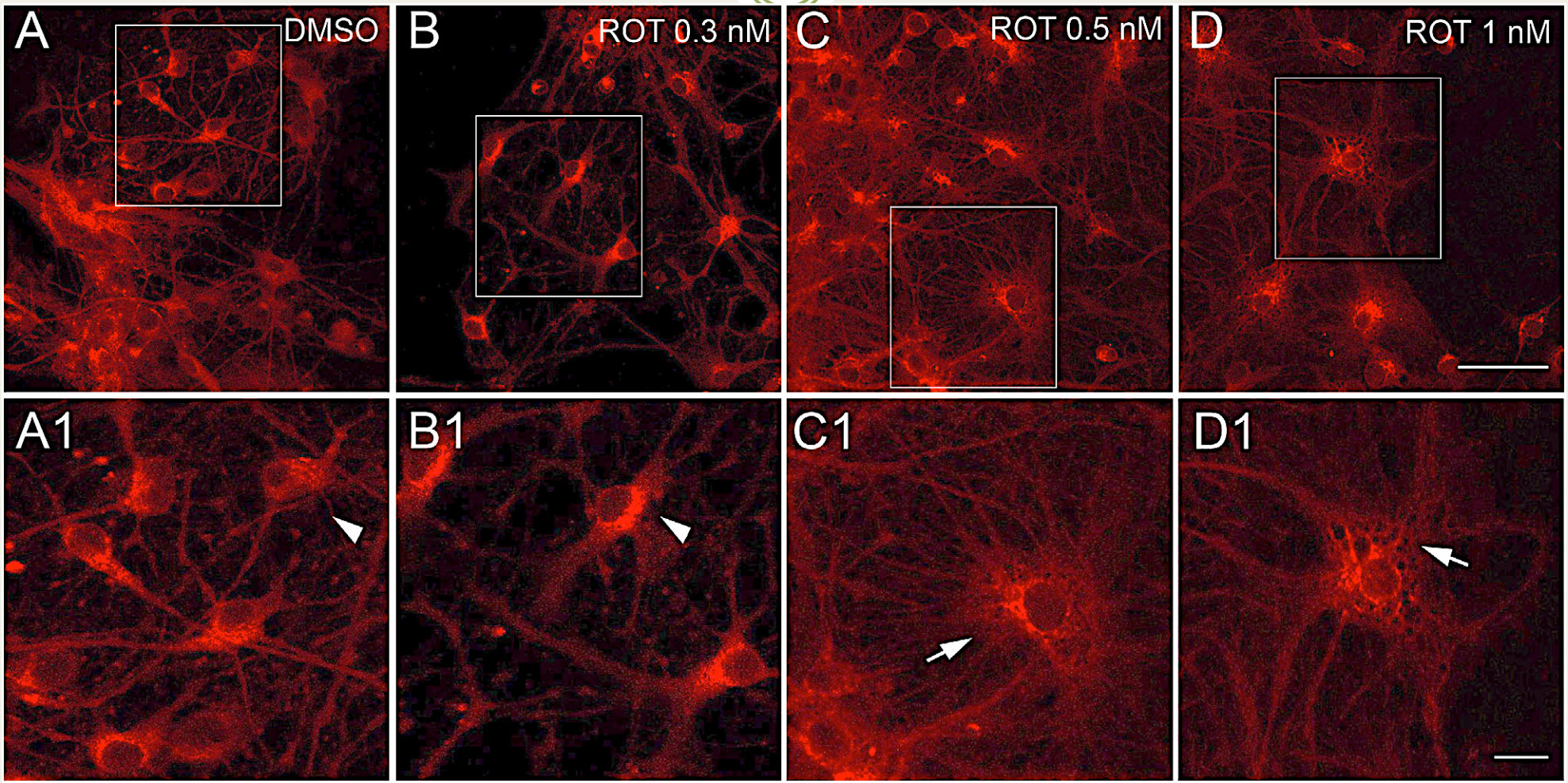
Rab1 transfection

Confocal
microscopy



- ✓ Inhibition of mitochondrial complex I
- ✓ Action upon neurons and glial cells
- ✓ Promotes protein aggregation
- ✓ Increases oxidative stress
- ✓ Mimics ageing

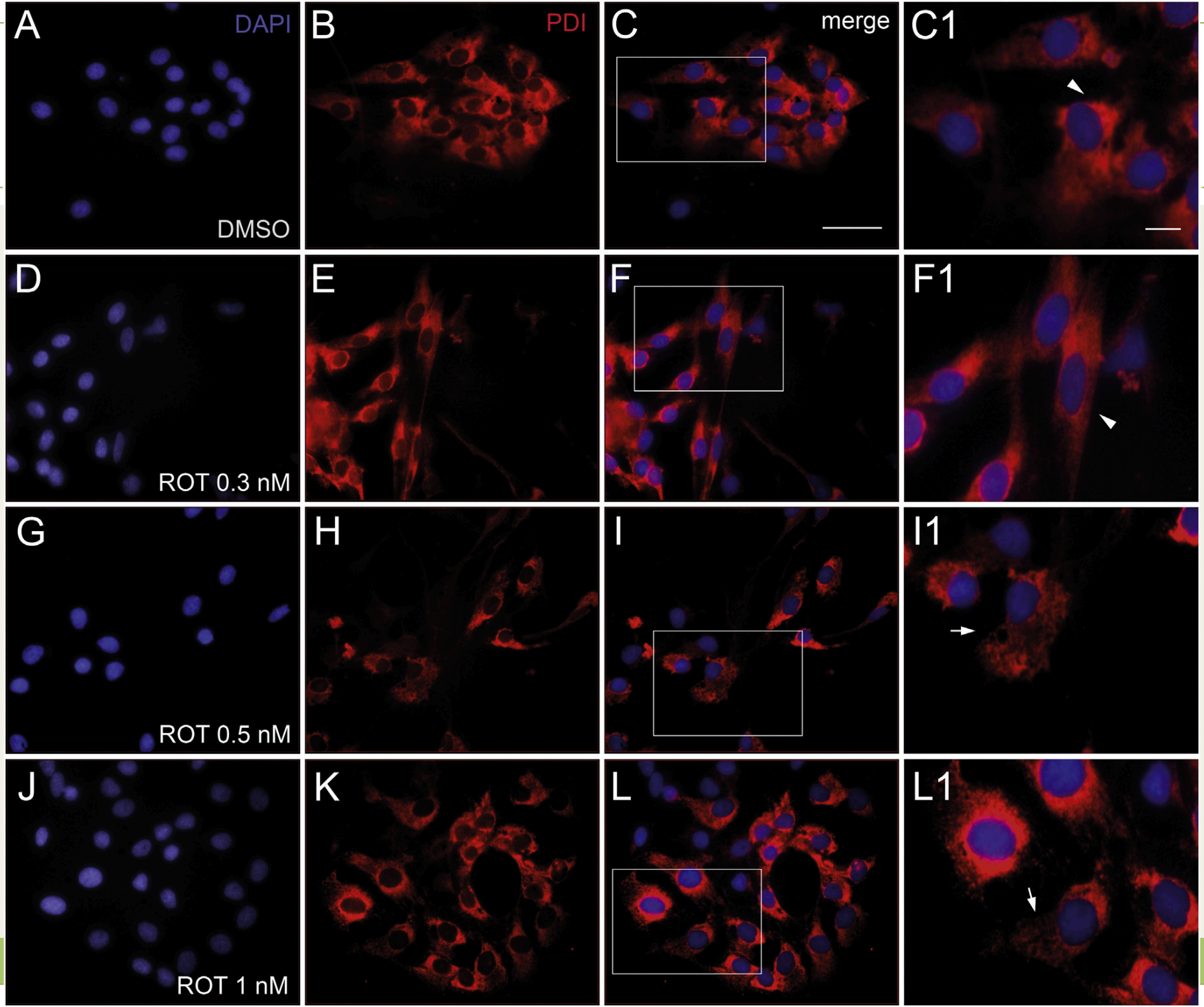
Results – ER Morphology



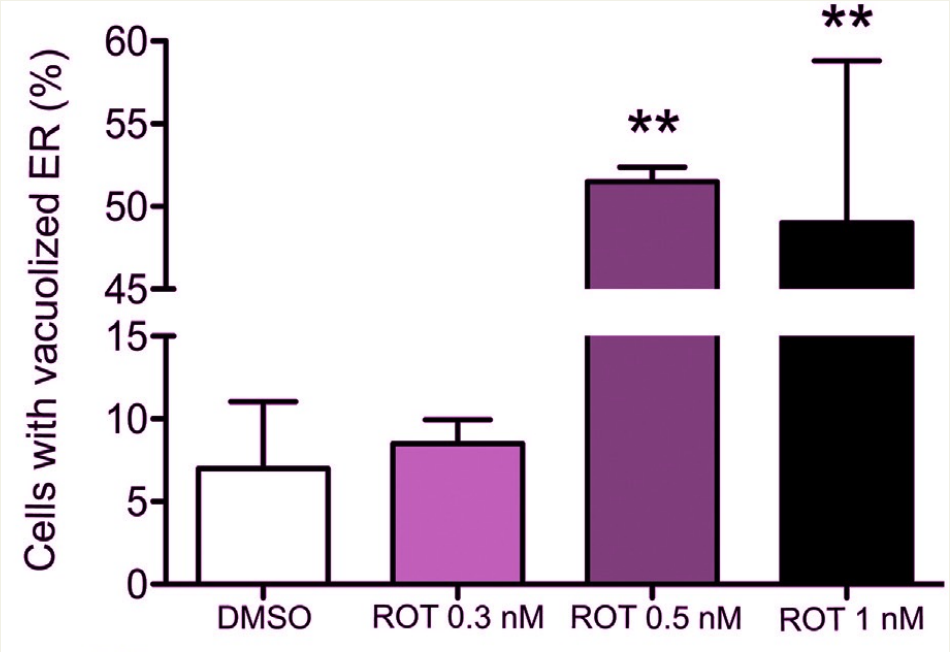
ER morphology changes from smooth (arrow heads) to vacuolized (arrows) in the presence of protein oligomers and aggregation.

Scale bar=50 μm for upper panels and 20 μm for lower panels.

Lima et al. Ferrari Neuroscience (2019) 419:5-13


A
DAPI
DMSO
B
PDI
C
merge
C1
D
ROT 0.3 nM
E
F
F1
G
ROT 0.5 nM
H
I
I1
J
ROT 1 nM
K
L
L1

Results – Quantification of ER vacuolization



50% of cells presented vacuolized ER during protein aggregation

One-way ANOVA **p<0.01 compared to DMSO. N=5

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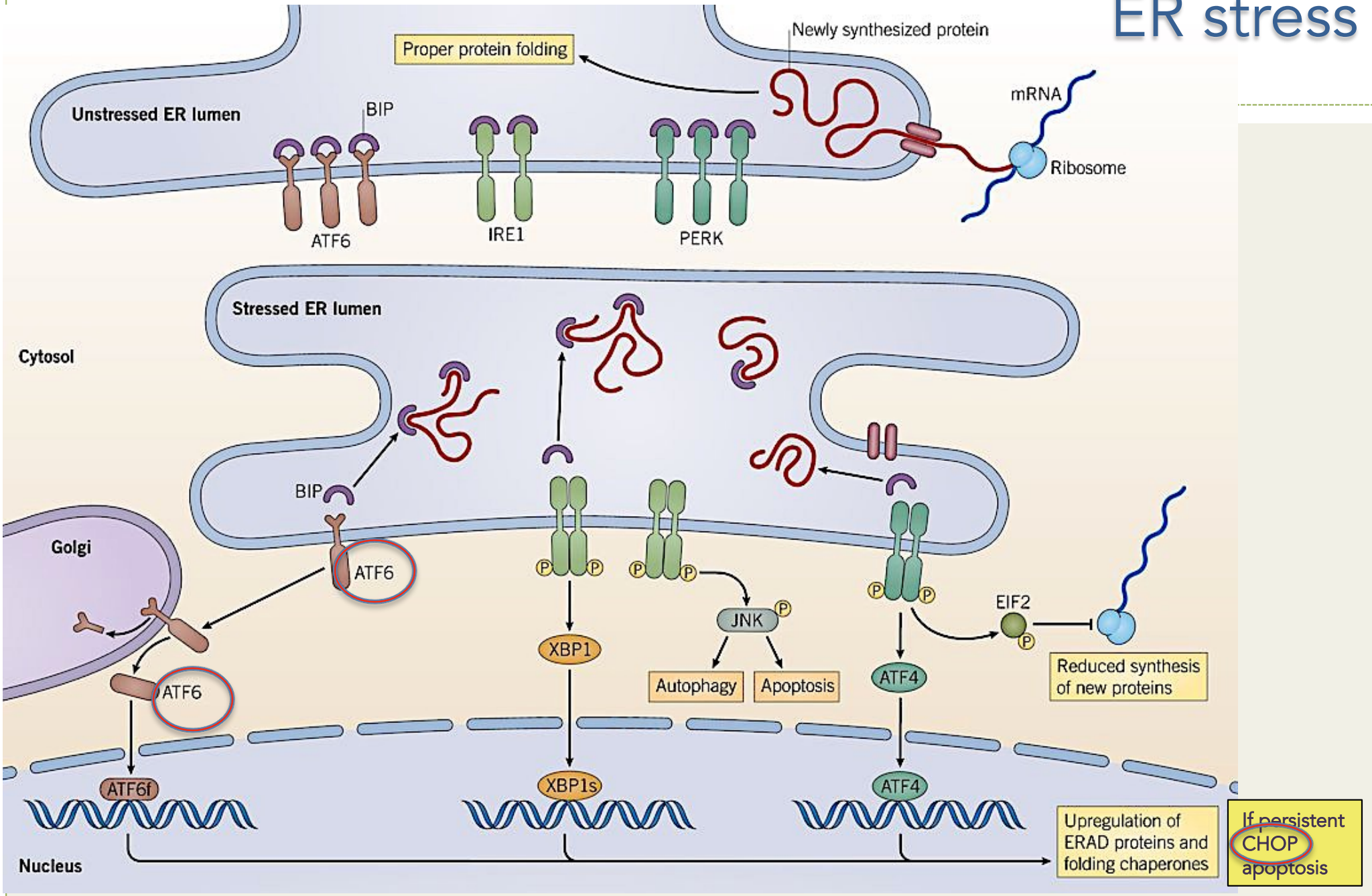
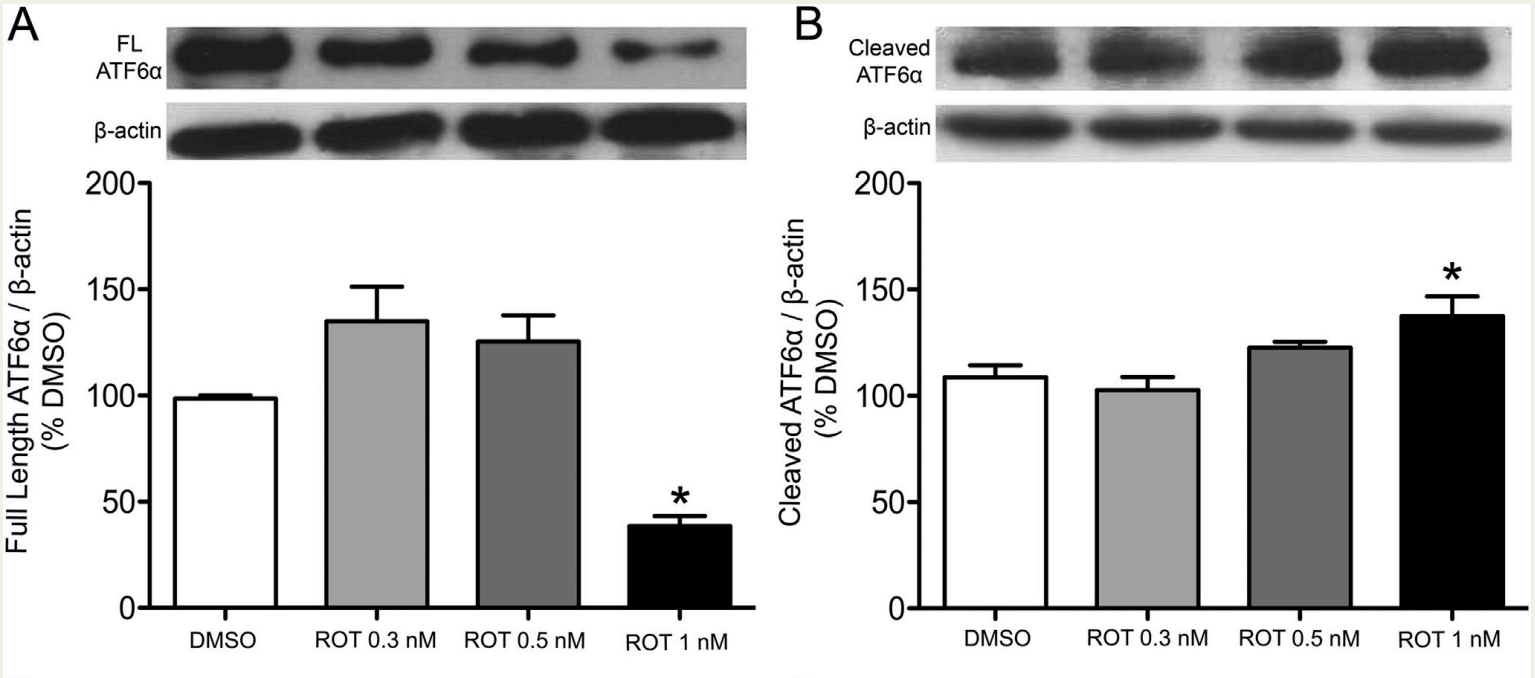
ER stress



Adapted from Todd et al. (2008) Nat.Rev.Immunol. 8 663

Results



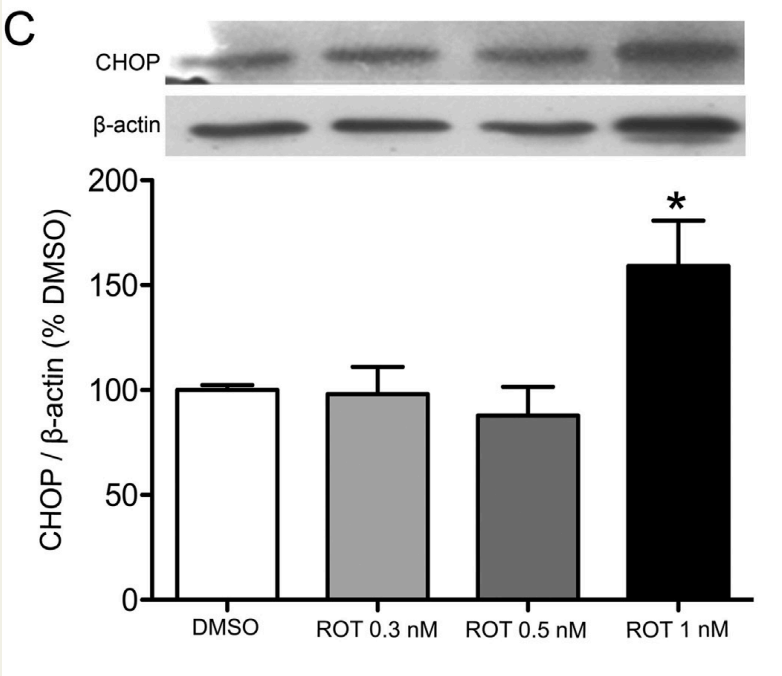
ER stress

Increased levels of cleaved ATF6 during protein aggregation

One-way ANOVA
*$p<0.05$ compared to DMSO. N=5

Lima et al. Ferrari Neuroscience (2019) 419:5-13

Results



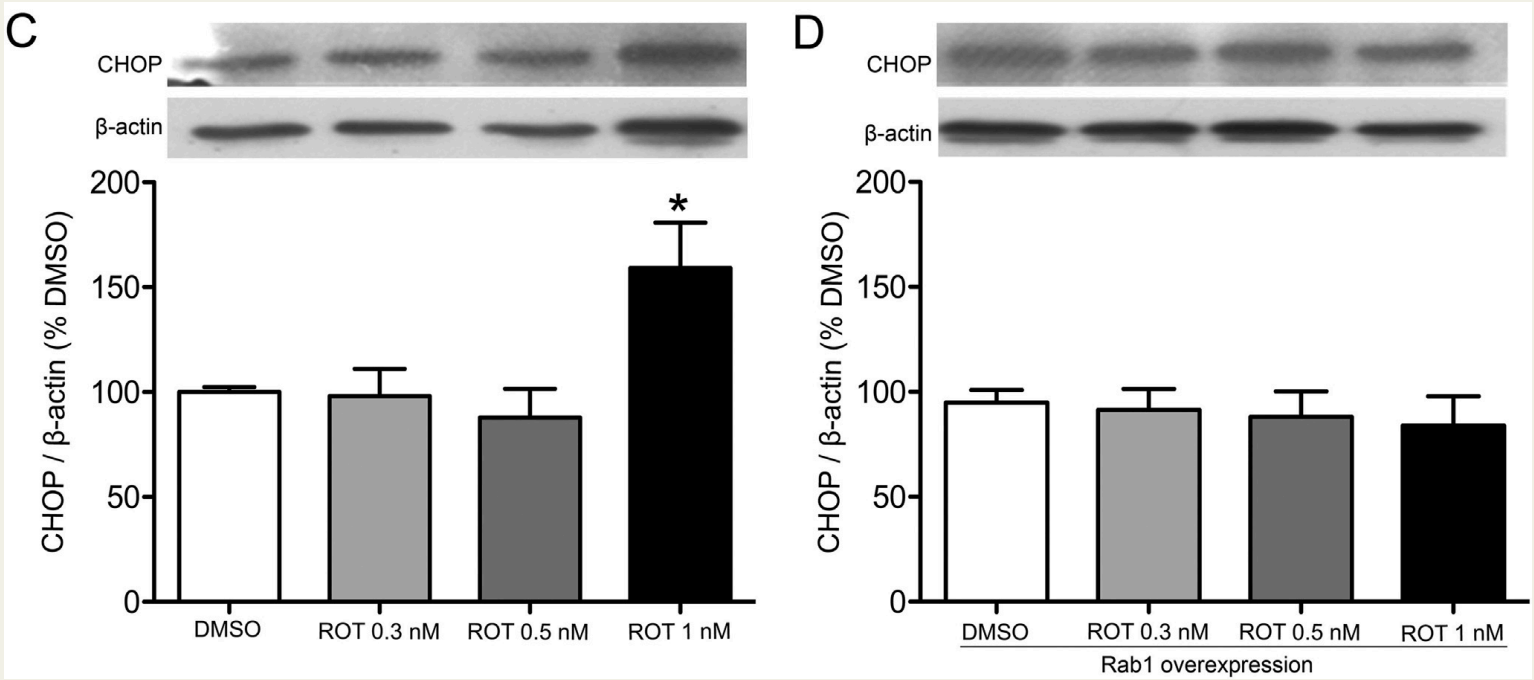
ER stress

Increased levels of CHOP expression during protein aggregation

One-way ANOVA
*p<0.05 compared to DMSO. N=5

Lima et al. Ferrari Neuroscience (2019) 419:5-13

Results



ER stress

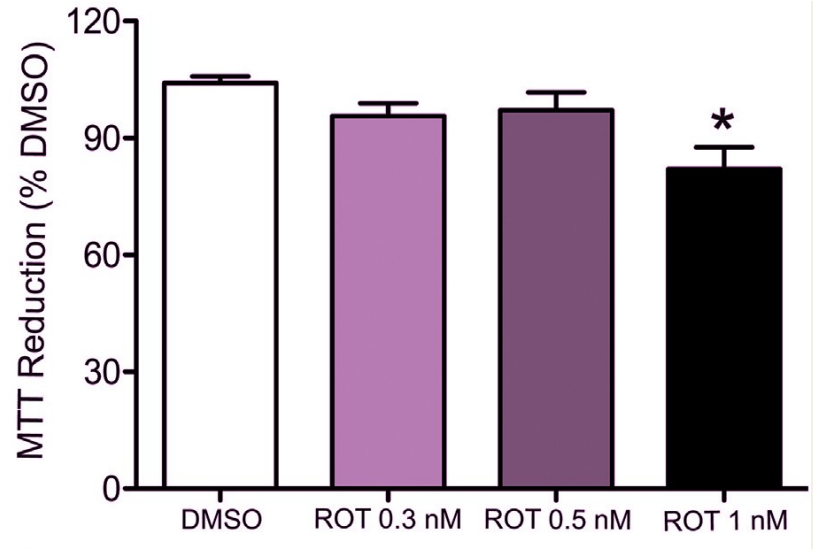
Increased levels of CHOP expression during protein aggregation

Rab1 overexpression prevents the increase in CHOP levels

One-way ANOVA
*$p<0.05$ compared to DMSO. N=5

Lima et al. Ferrari Neuroscience (2019) 419:5-13

Results – cell viability

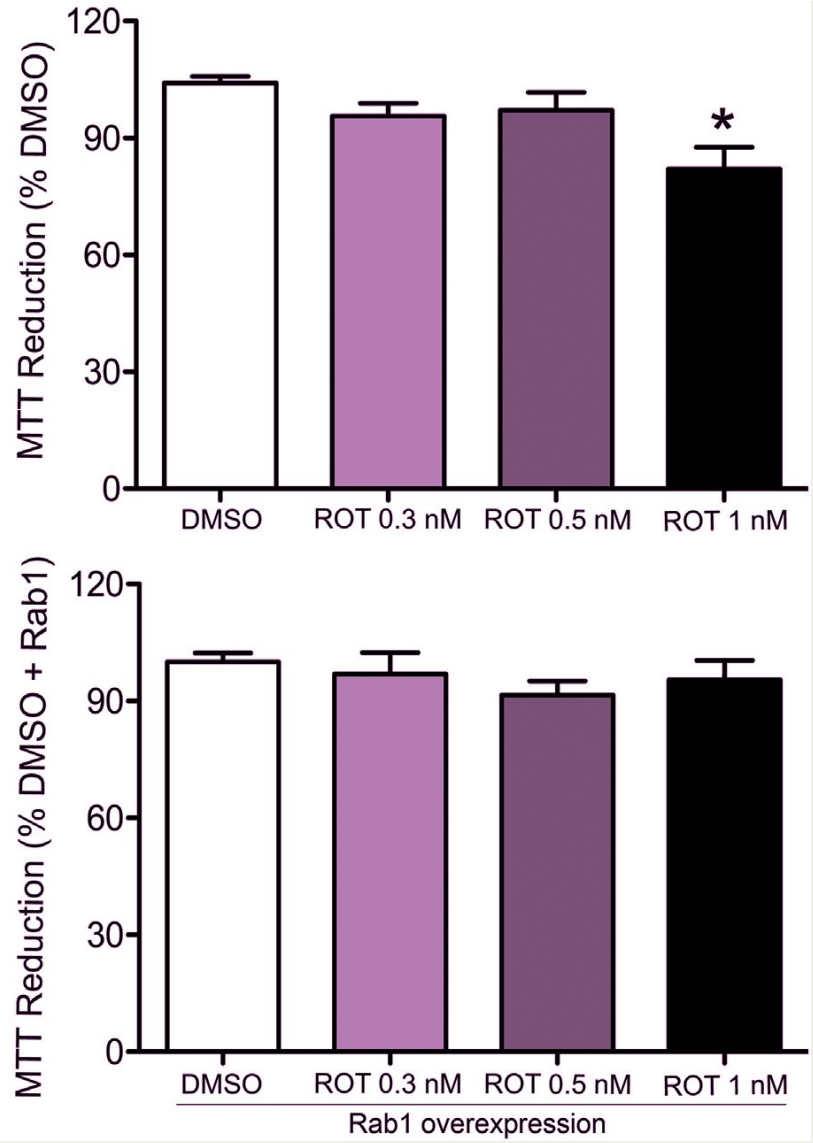


Decrease in cell viability in the presence of protein aggregation agrees with increased CHOP levels

One-way ANOVA *$p<0.05$ compared to DMSO. N=5

Lima et al. Ferrari Neuroscience (2019) 419:5-13

Results – cell viability



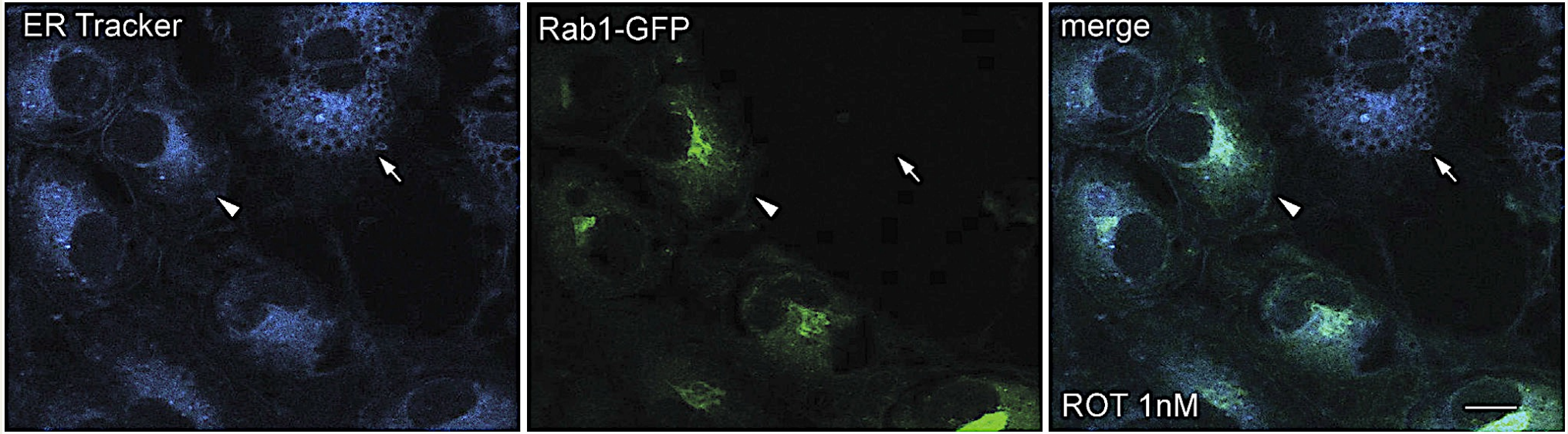
Decrease in cell viability in the presence of protein aggregation agrees with increased CHOP levels

Rab1 overexpression prevents cell death

One-way ANOVA *$p<0.05$ compared to DMSO. N=5

Lima et al. Ferrari Neuroscience (2019) 419:5-13

Results – ER vacuolization and Rab1



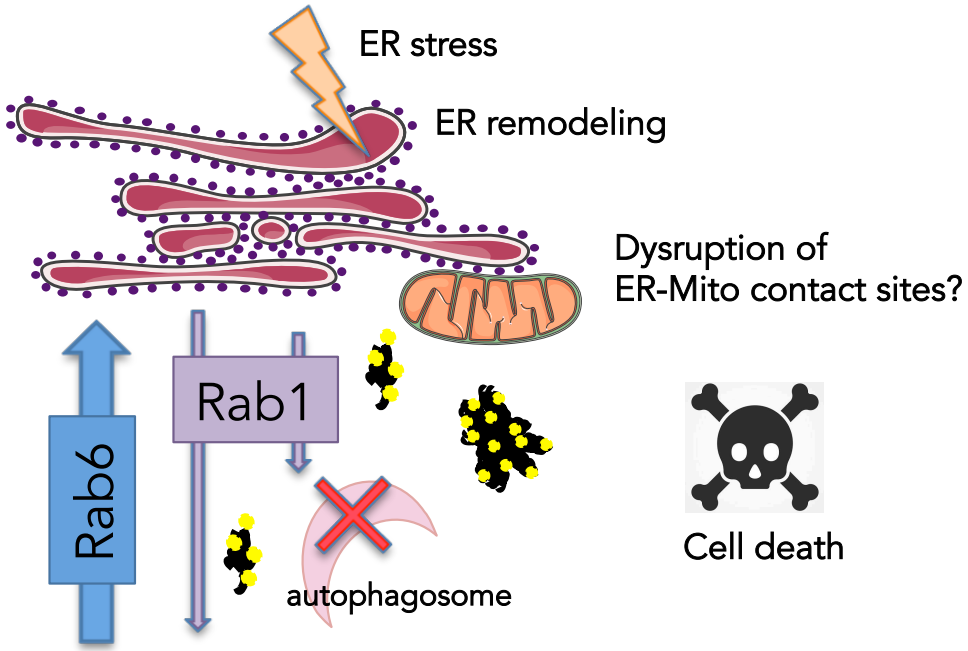
Cells expressing Rab1 present compacted ER (arrow heads)

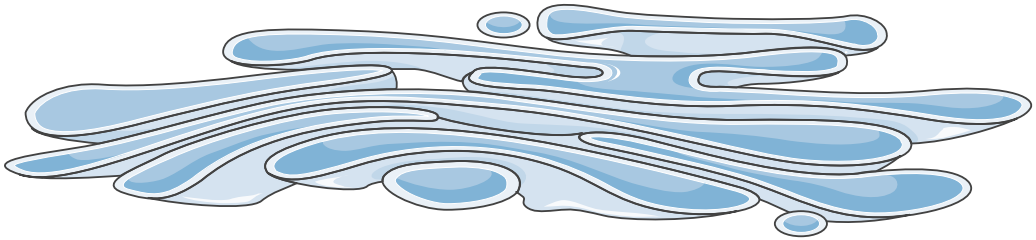
Cells lacking Rab1 show vacuolized ER (arrows)

Scale bar=50 μm for upper panels and 20 μm

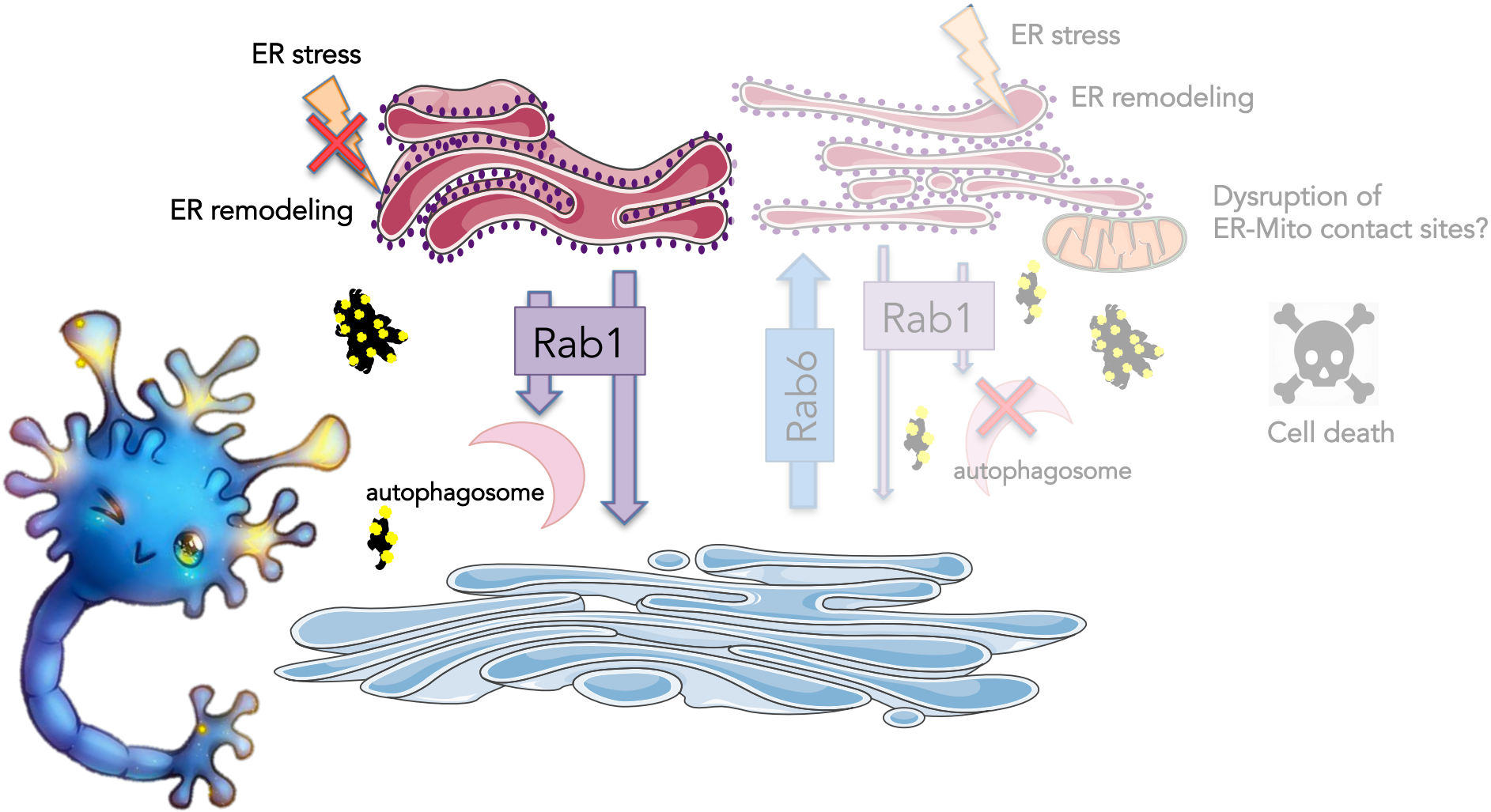
Lima et al. Ferrari Neuroscience (2019) 419:5-13

Summary


ER stress
ER remodeling
Dysruption of
ER-Mito contact sites?
Rab1
Rab6
autophagosome
Cell death



Summary


ER stress
ER remodeling
Rab1
autophagosome
ER stress
ER remodeling
Dysruption of
ER-Mito contact sites?
Rab6
Rab1
autophagosome
Cell death

Acknowledgements

Current Lab members

Former Lab members who were involved in this study

CEPID
Centro de Pesquisa sobre o Genoma Humano e Células-Tronco
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
CAPES
FAPESP
USP