SCIENCES FONDAMENTALES
ET BIOMÉDICALES
Sciences
Université Paris Cité

From Prions to Alzheimer's disease: contribution of the 1C11 neuronal cell line

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Mad cow disease= example of prion diseases

Mad cow crisis (1988-2003):

GB : 185 000 cases of mad cow

France : 1000 cases of mad cow

Transmissible Spongiform Encephalopathies (TSE)
= class of neurodegenerative disorder

Human

Fatal Familial Insomnia

KURU

Creutzfeldt-Jakob Disease And variant

Gertsmann-Sträussler-Scheinker Disease

PRIONS

Scrapie (sheep)

Chronic Wasting Disease (Cervids)

BSE (cow)

Transmissible mink Encephalopathy

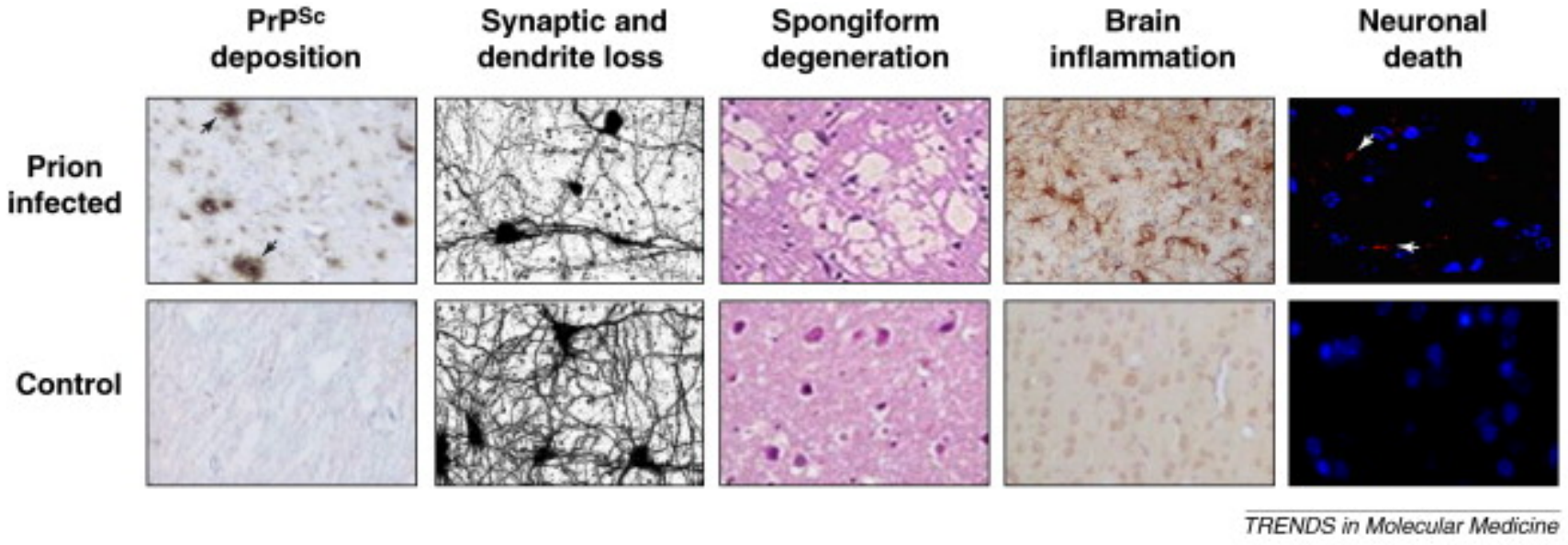
Animal

Features of TSEs

- ✓ TRANSMISSIBLE**:** naturally e.g. mad cow or experimentally
- ✓ SPONGIFORM: Vacuoles
- ✓ ENCEPHALOPATHY : Neuronal death (apoptosis) => brain atrophy

+

- ✓ Amyloid plaques : Protein misfolding and aggregation
- ✓ Astrogliosis = activation of glial cells (as a cause or a consequence of inflammation)



Definitive diagnostic : biopsy of brain tissue (post mortem)

(Soto et Satani, 2011)

Another hallmark of prion disease: Amyloid plaques

Prion disease: both intracellular and extracellular accumulation of amyloid aggregates = plaques similar to those characteristic of AD, and positive to prion protein staining.

Alzheimer's | Creutzfeldt-Jakob's

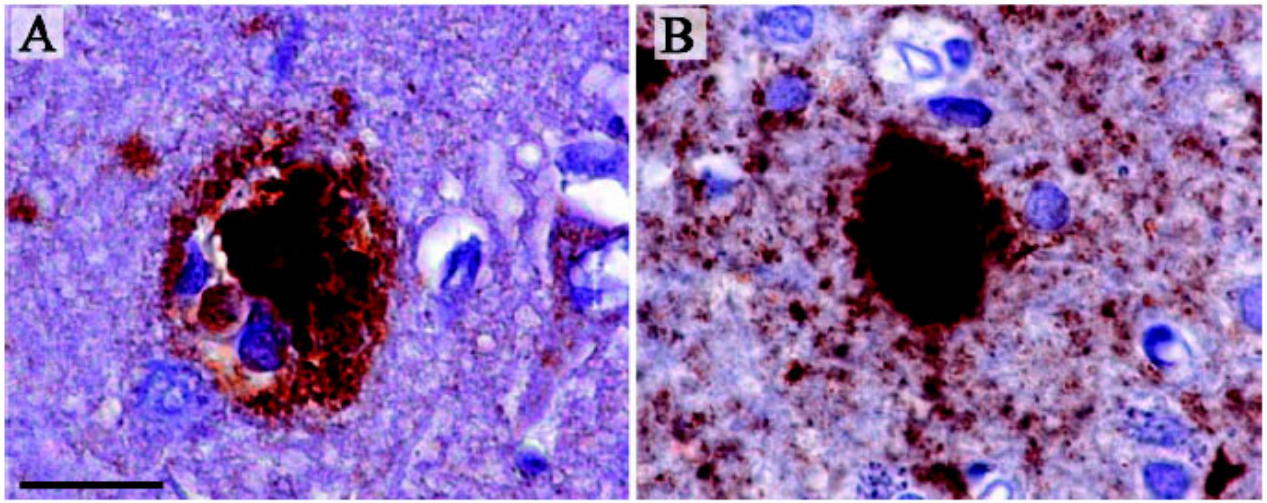


Fig. 1. Both Alzheimer's and prion diseases are characterized by the deposition of pathological proteins in the brain, often in the form of plaques. The brown color is indicative of immunostained cortical deposits of the Aβ peptide and of the PrP^{Sc} protein in brains of patients suffering from Alzheimer's disease (**A**) and Creutzfeldt-Jakob disease (**B**), respectively. Scale bar: 20 μm.

Aguzzi A, Haass C. Science. 2003 Oct 31;302(5646):814-8. Review.

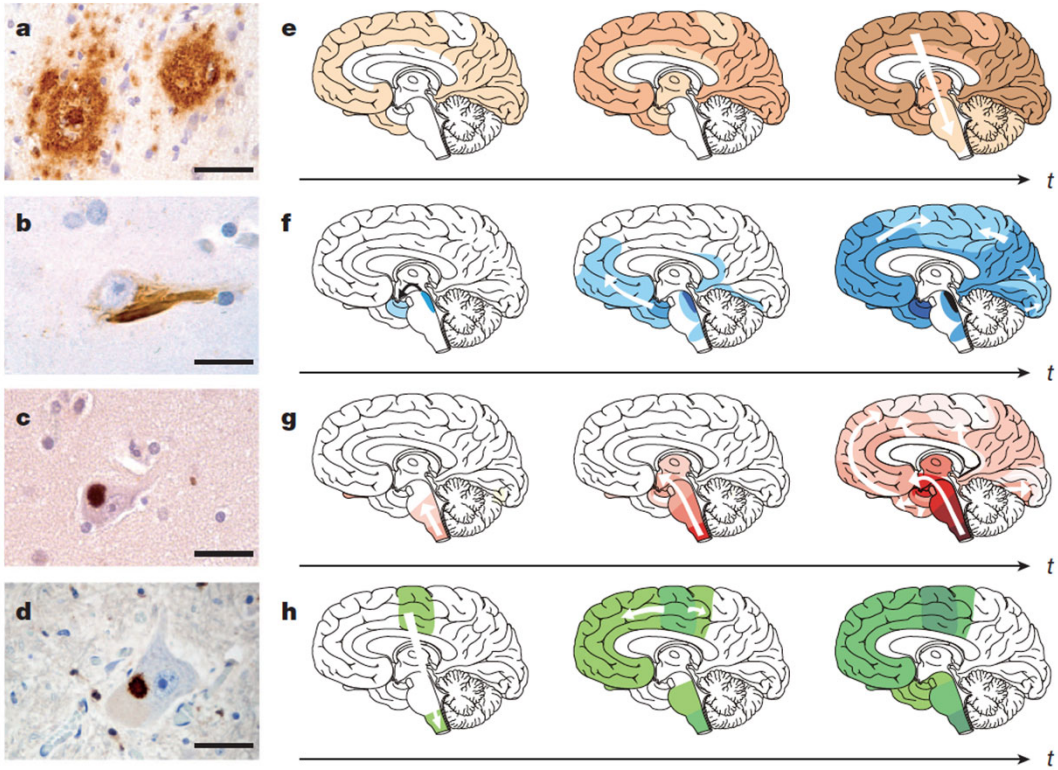
TSEs share common features with other neurodegenerative diseases

Neuronal cell death, Age-related diseases, Aggregation of particular proteins
= proteopathies = amyloid-based neurodegenerative diseases.

Alzheimer
- Plaque senile ← Aβ
- NFT ← Tau

Parkinson — α-synuclein

Amyotrophic Lateral sclerosis — TDP-43

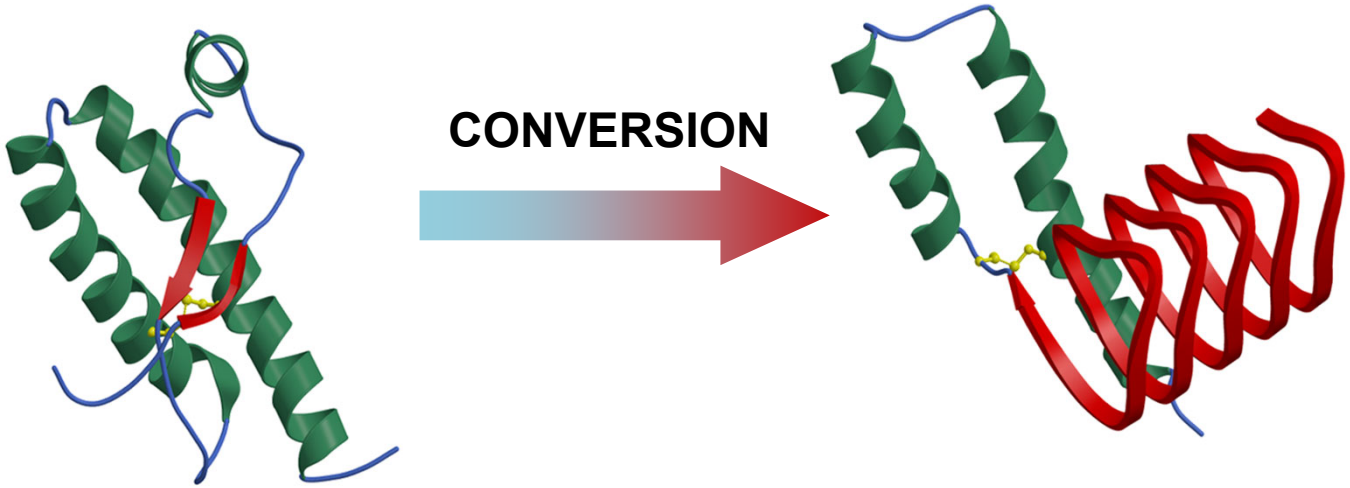


Jucker M. Nature,2013

BUT: location and pattern of protein aggregation ≠
Prion diseases are the sole infectious neurodegenerative disease

Prion concept

- S. Prusiner (Nobel Prize 1997) dropped a dogma of biology:
  one protein can be infectious => Proteinacenous Infectious particle



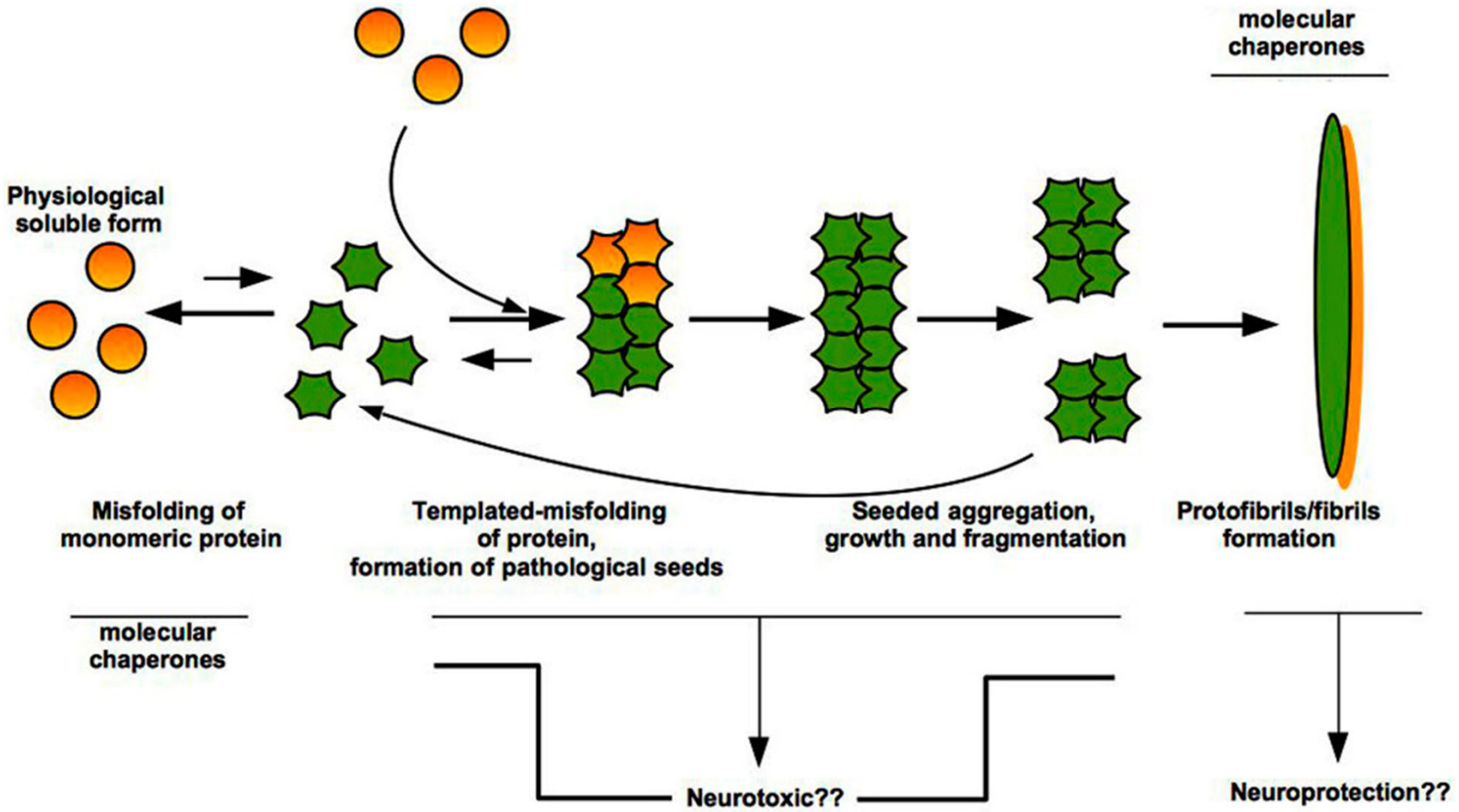
Cellular prion protein
(PrP^{C})

Scrapie prion protein
(PrP^{Sc})

- ✓ Normal protein of the host
- ✓ Ubiquitous protein (more expressed in neurons)

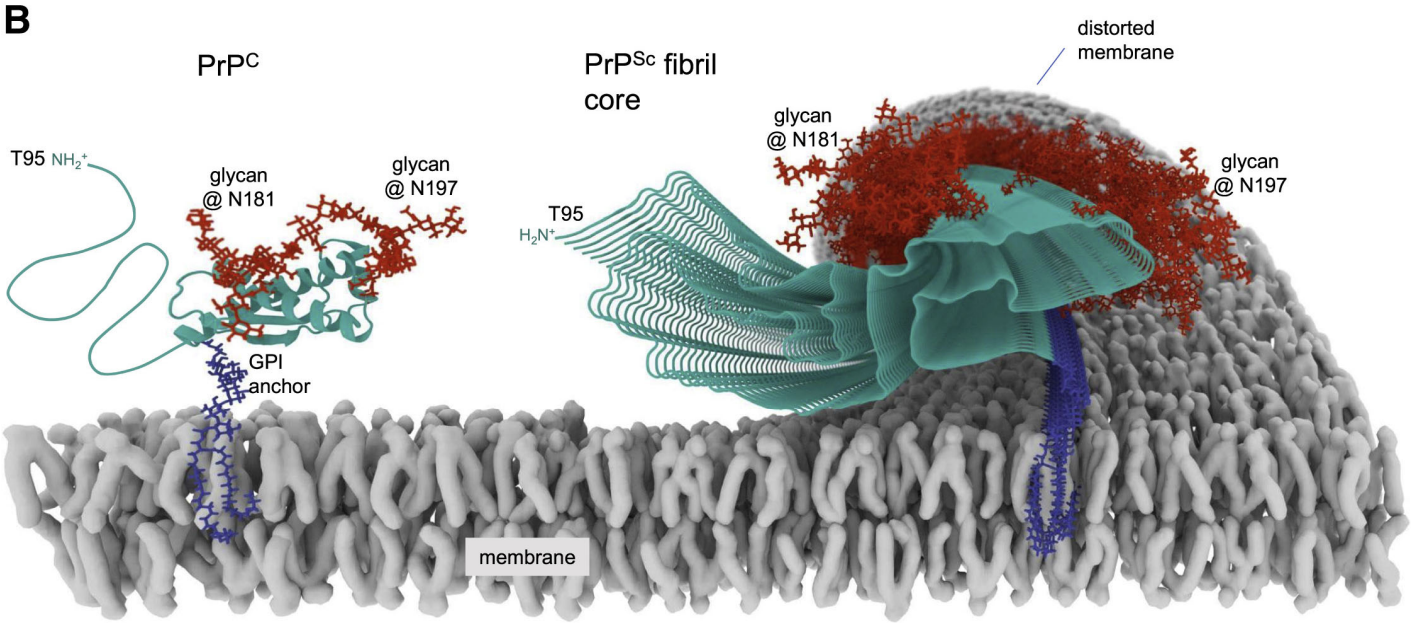
- ✓ Main component of Prions
- ✓ Only in TSE-afflicted brains
- ✓ Enriched in β sheets

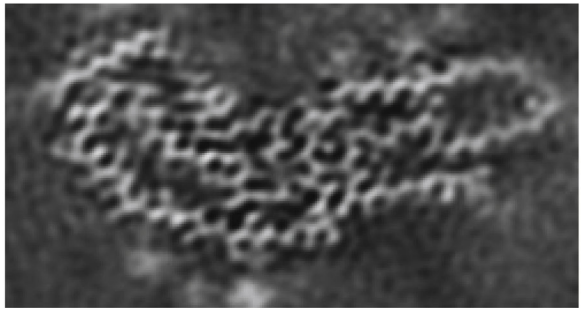
How prions propagate?
Nucleation polymerization model for $PrP^{C} \rightarrow PrP^{Sc}$ conversion.



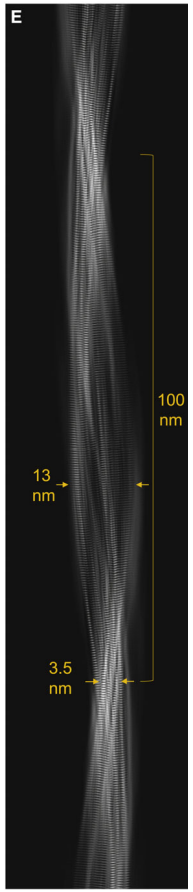
=> Prion-like proteins : Aβ, Tau, α-synuclein…

How prions propagate?
Models for $PrP^{C} \rightarrow PrP^{Sc}$ conversion.





Projection of density map of fibril cross-section derived from single-particle cryo-EM analysis



Projection of the fibril density map

*High-resolution structure and strain comparison of infectious mammalian **prions**.*
Kraus A, et al. Mol Cell. 2021 doi: 10.1016/j.molcel.2021.08.011.

Why prion-infected neurons die?

- PrP KO mice are resistant to infection by pathogenic prions

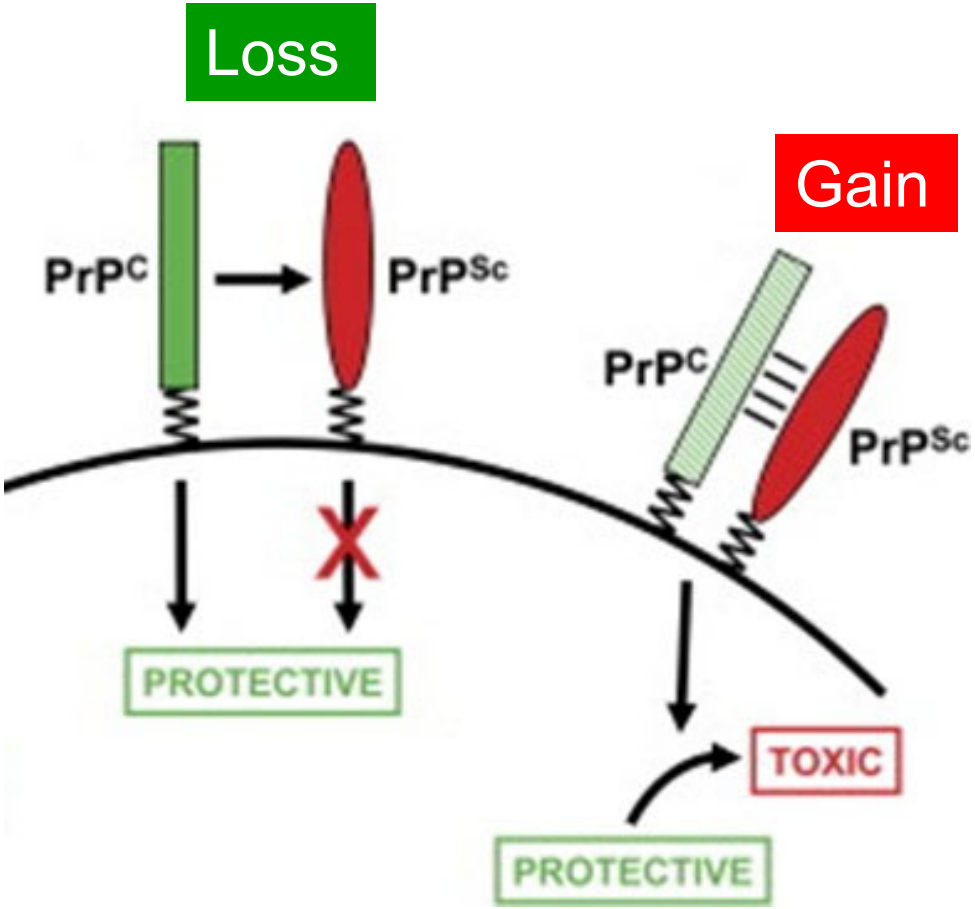
Depleting Neuronal PrP in Prion Infection Prevents Disease and Reverses Spongiosis

Giovanna Mallucci, Andrew Dickinson, Jacqueline Linehan, Peter-Christian Klöhn, Sebastian Brandner, John Collinge*

The mechanisms involved in prion neurotoxicity are unclear, and therapies preventing accumulation of PrP^{Sc}, the disease-associated form of prion protein (PrP), do not significantly prolong survival in mice with central nervous system prion infection. We found that depleting endogenous neuronal PrP^{C} in mice with established neuroinvasive prion infection reversed early spongiform change and prevented neuronal loss and progression to clinical disease. This occurred despite the accumulation of extraneuronal PrP^{Sc} to levels seen in terminally ill wild-type animals. Thus, the propagation of non-neuronal PrP^{Sc} is not pathogenic, but arresting the continued conversion of PrP^{C} to PrP^{Sc} within neurons during scrapie infection prevents prion neurotoxicity.

=> It is necessary to understand PrP^{C} function to decipher mechanisms of prions neurotoxicity!

Why prion-infected neurons die?
Corruption of PrP^C function

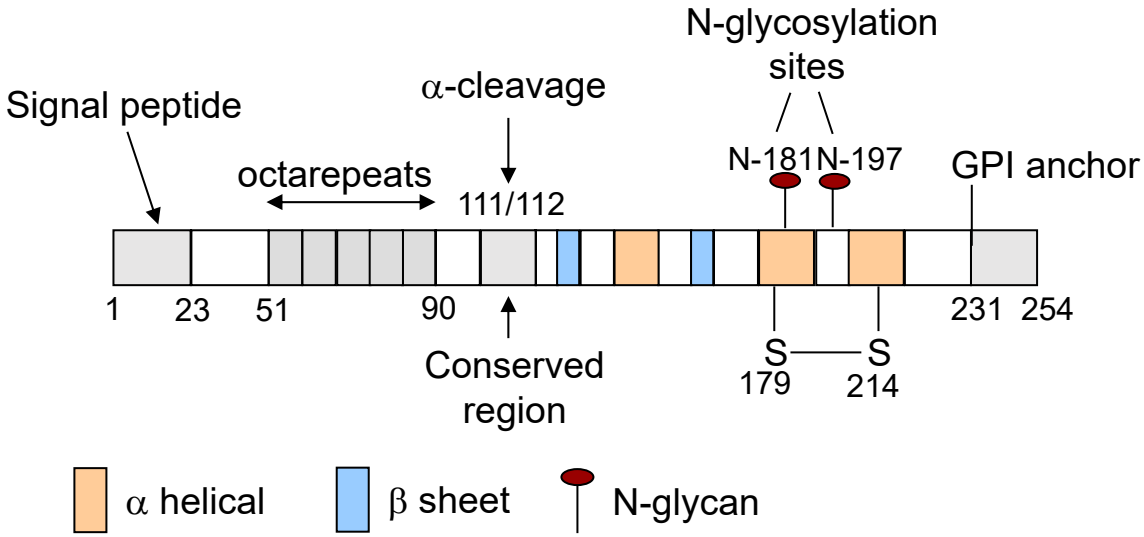


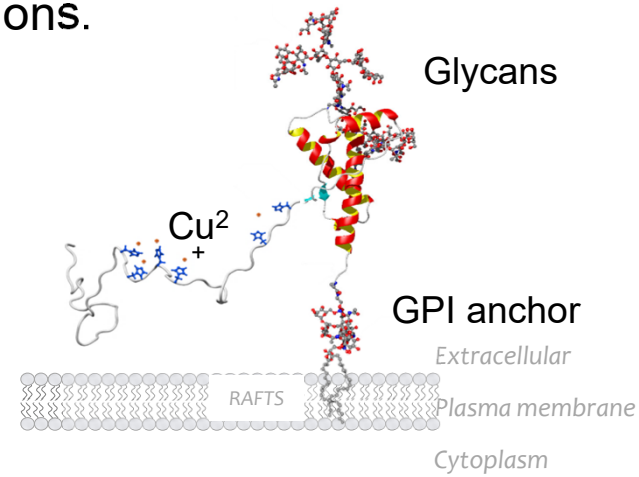
=> It is necessary to understand PrP^C function to decipher mechanisms of prions neurotoxicity!

(Harris et al, 2006)

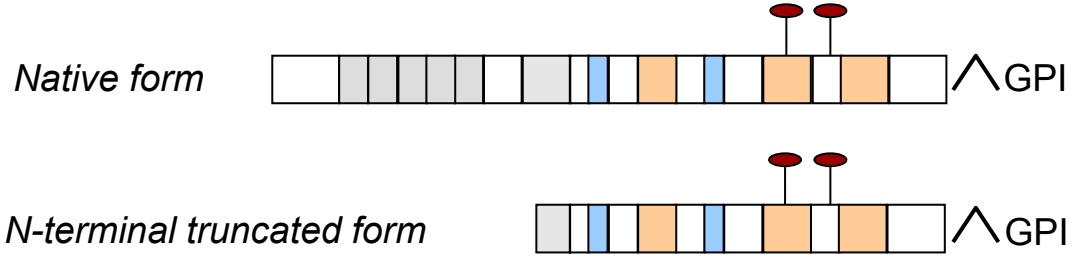
Cellular Prion Protein

- Primary structure and post-translational modifications.

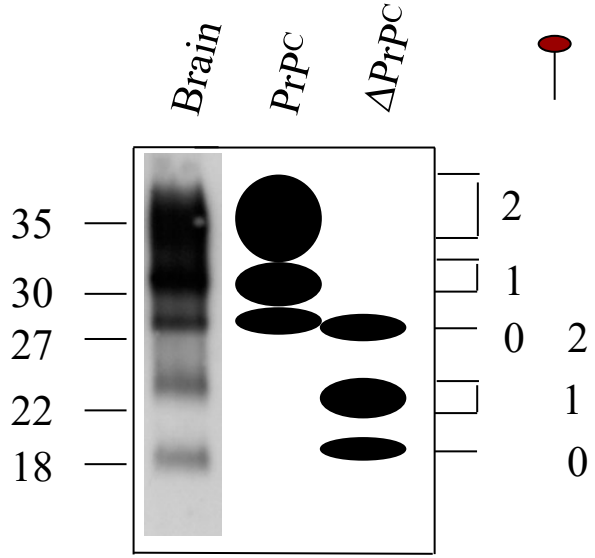




- Glycosylation and binding to metals



✓ α-Cleavage



=> Not 1 PrP^C but various isoforms

Physiologic role of Cellular Prion protein

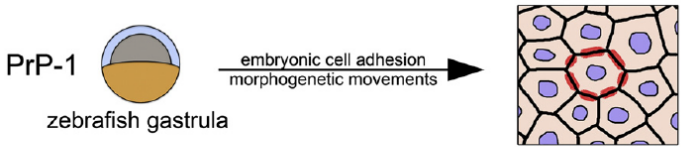
$PrP^{-/-}$ models:

- **souris**

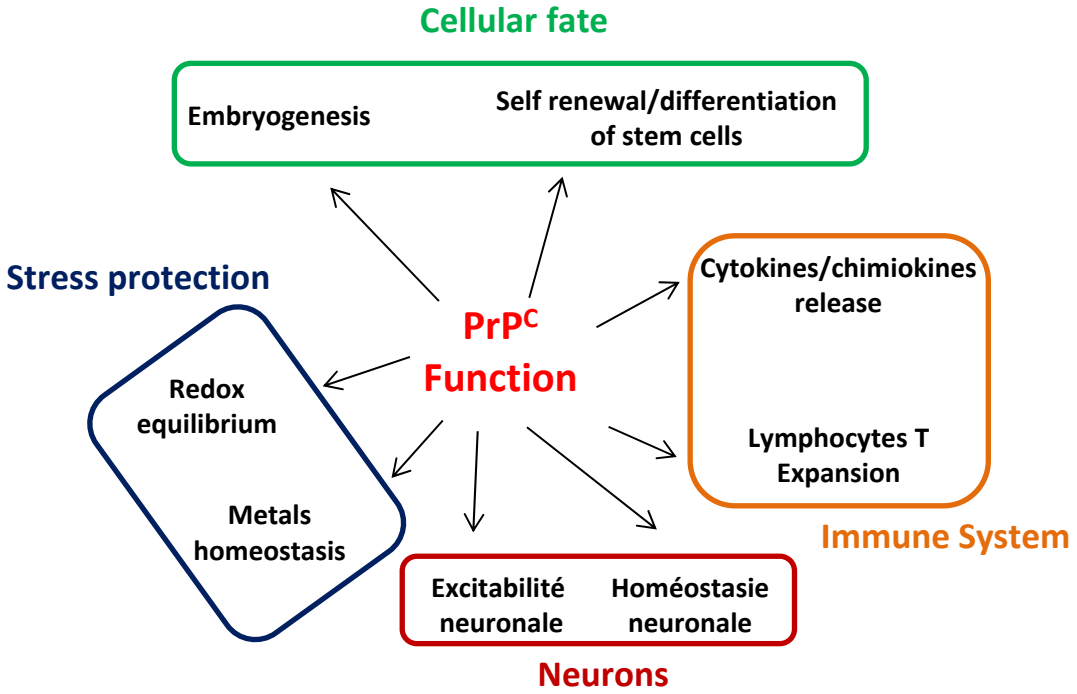
Viables & normal CNS development

=> No obvious function for PrP^C

- **Zebra Fish**



Málaga-Trillo 2011; Leighton 2018



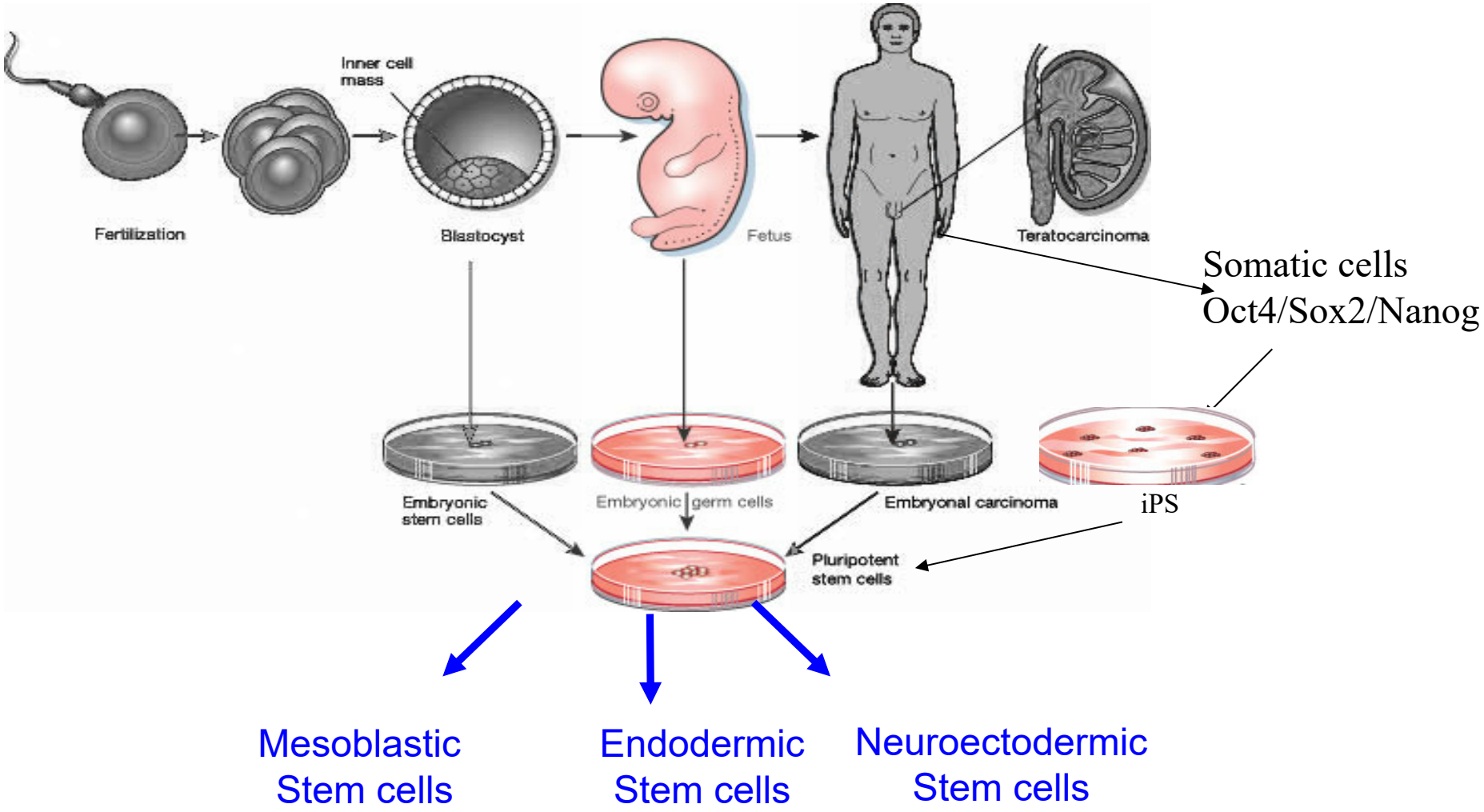
Ubiquitous role

Neurospecific role

⇒ No obvious function for PrP^C ! Protective role?

⇒ Hyp: PrP^C is so important for neuronal cell homeostasis that mechanisms compensate lack of PrP^C

Pluripotent stem cells: ES/EG/EC/iPS

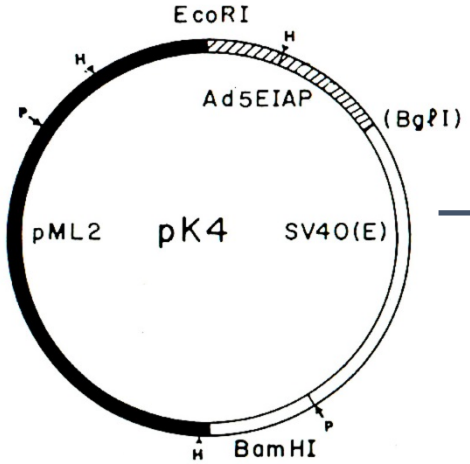


Cell populations obtained after ES/EC/iPS differentiation are heterogeneous and stop dividing

➔ difficult to clone and select cell lines having properties of lineage precursor cells.

Strategy to select lineage precursor cells

pK4 pluripotent cell lines EC



pK4 transgenic mice

Induction of differentiation

3 germ layers derivatives
Differentiated cells + immature cells

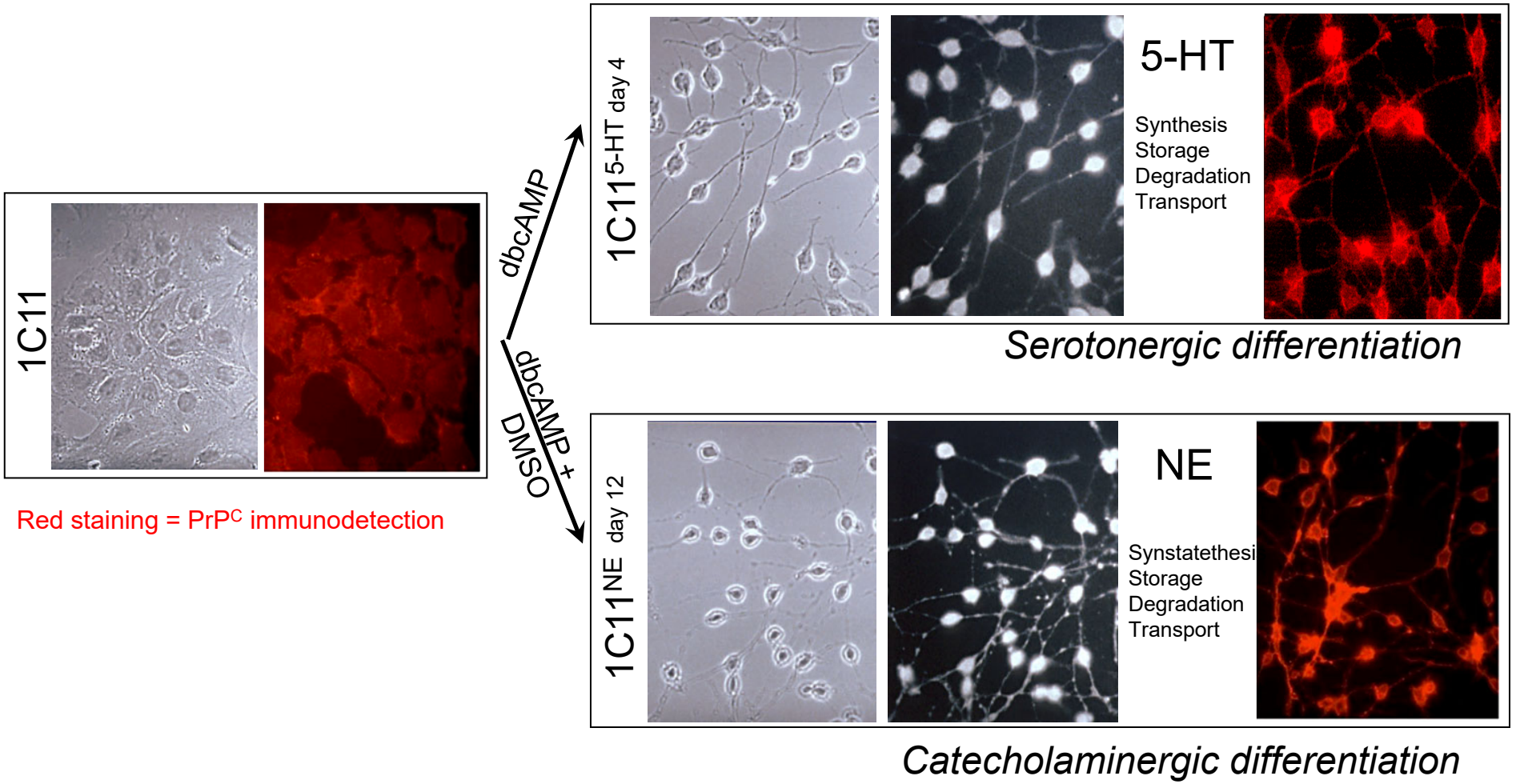
Cloning and Selection :
-loss of EC/ES cell markers
-Ability to differentiate at high frequency towards alternative fates along a given lineage

1C11 neuroectodermic cell line
C1 mesodermic cell line

Salivary gland cell lines
Renal cell lines

DENTAL Pulp stem cell lines
(ED18 molar of Tg embryos)

The 1C11 neuroectodermal cell line



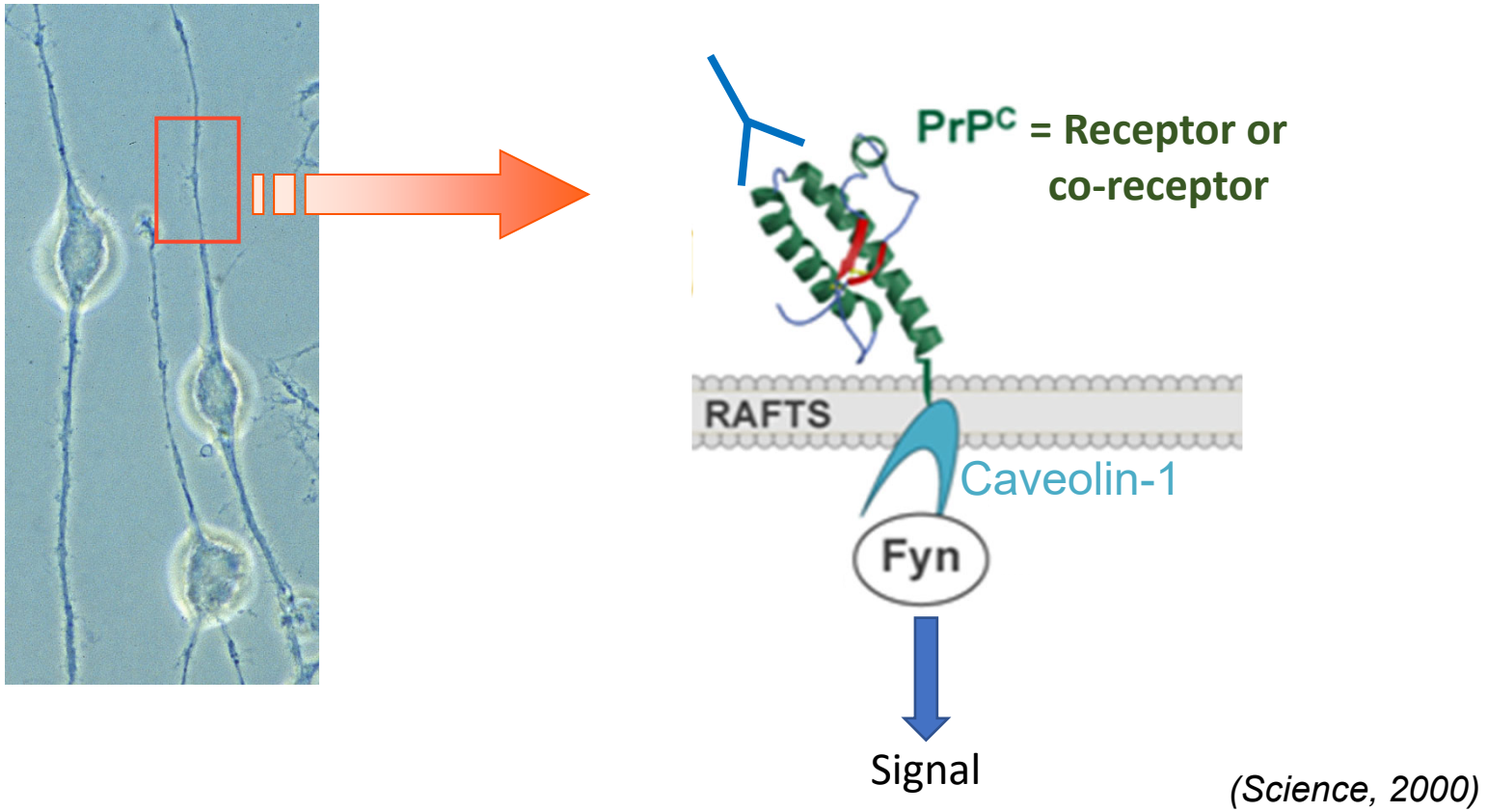
Red staining = PrP^C immunodetection

=>3 ≠ differentiation state, 100% differentiation

=> Expressed all PrP^C isoforms whatever differentiated state!

Signaling function of PrP^C
Proof of concept

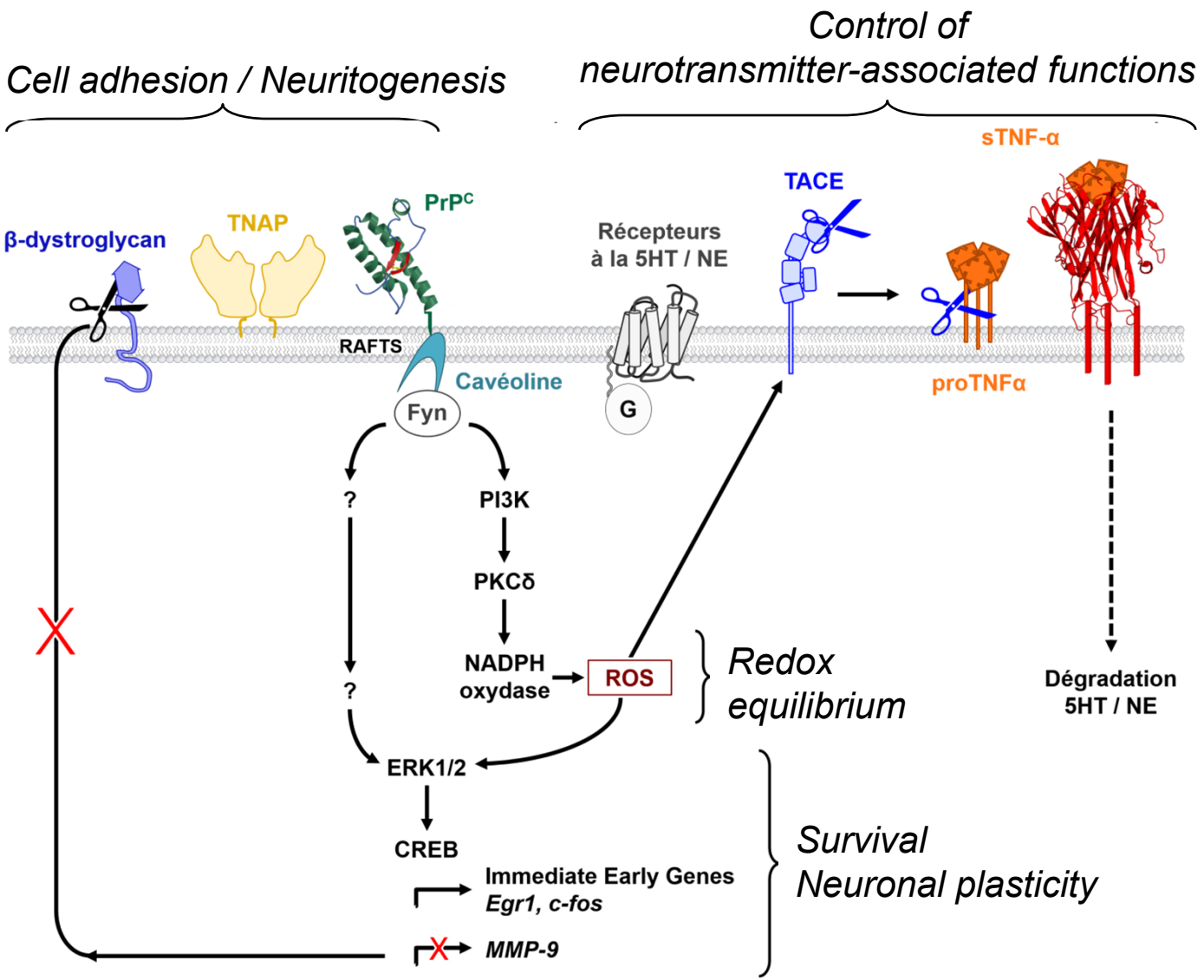
- Experimental strategy: cross linking using antbodies to mimic a natural ligand.



(Science, 2000)

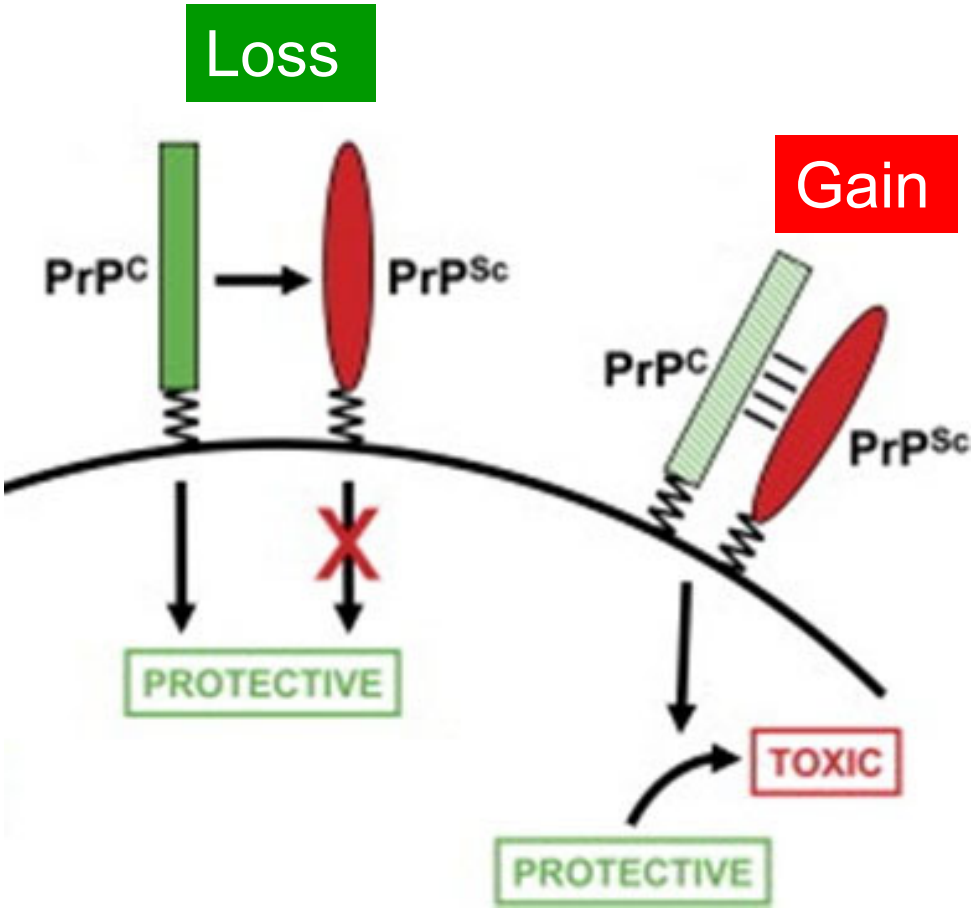
=> PrP^C-Caveolin1-Fyn Tyrosine kinase : Neurospecific signaling platform recruited only in neurites.

The multifaceted function of PrP^C in a neuronal context



Schneider et al. , Front. In Biosc., 2011

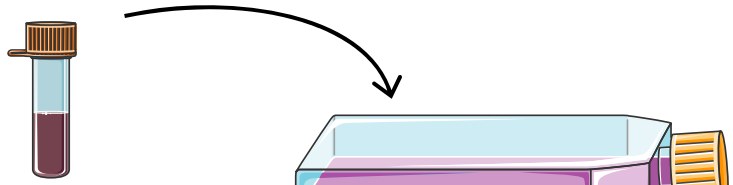
What is the impact of PrP^{Sc} on function of PrP^{C}? Loss / Gain?



(Harris et al, 2006)

What is the impact of PrP^{Sc} on function of PrP^{C}? Infection of 1C11 cell line

- The 1C11 cell line is chronically infected by several prion strains

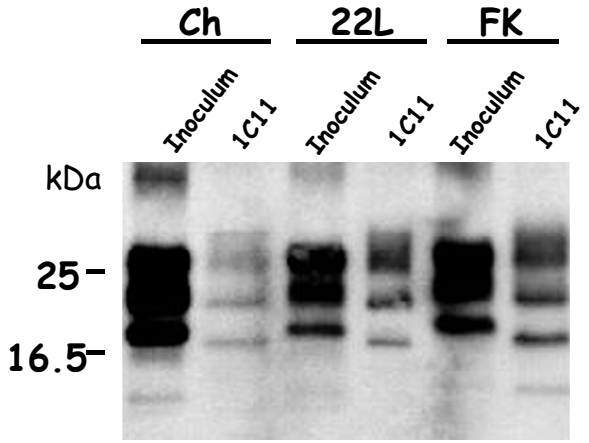


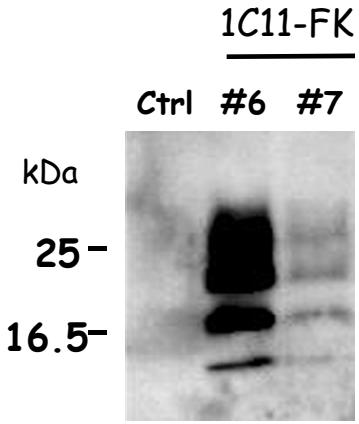
Infected brain homogenates (Chandler, 22l or FK)

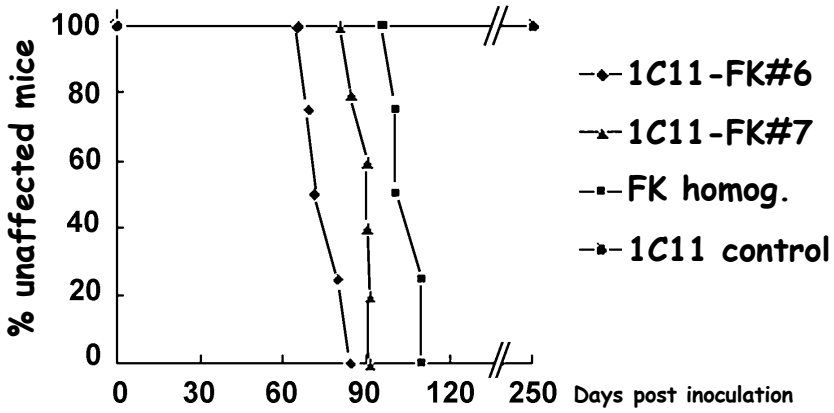
1C11 cells

1 passage → subcloning →

- PrP^{Sc} detection
- Bioassay



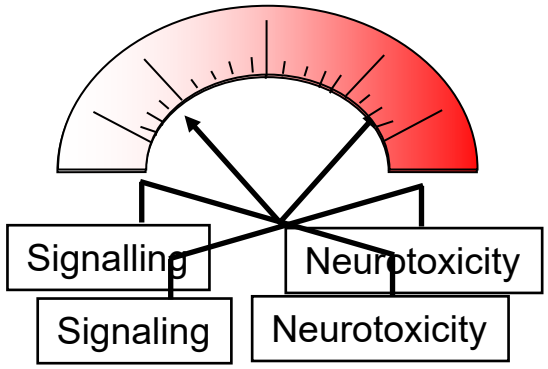




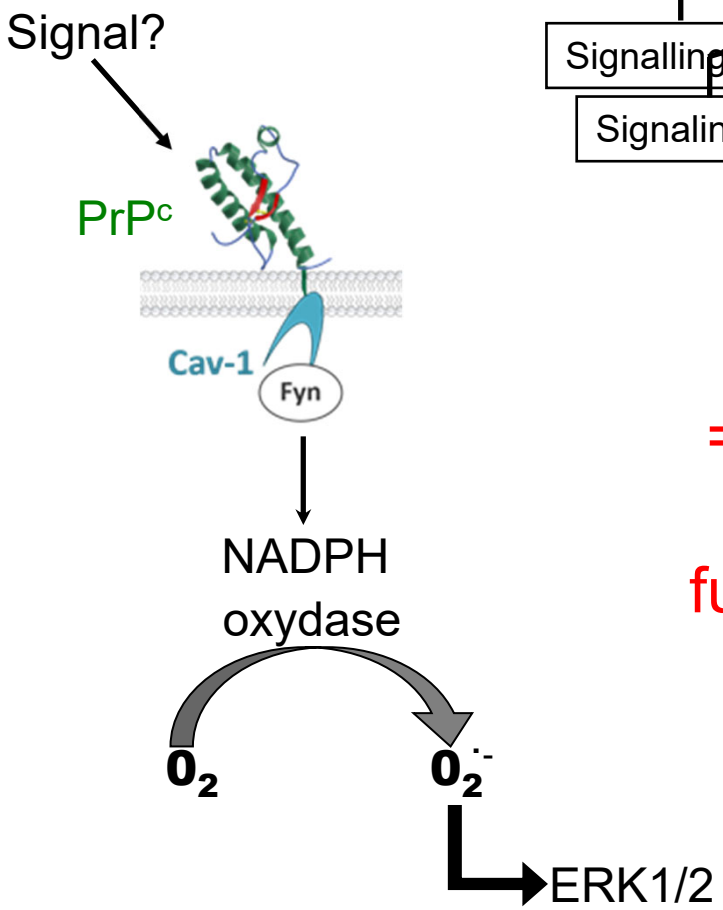
(Mouillet-Richard S et al., JBC, 2008)

=> Status of PrP^{C}–coupled signaling pathway in prion infected 1C11 cells?

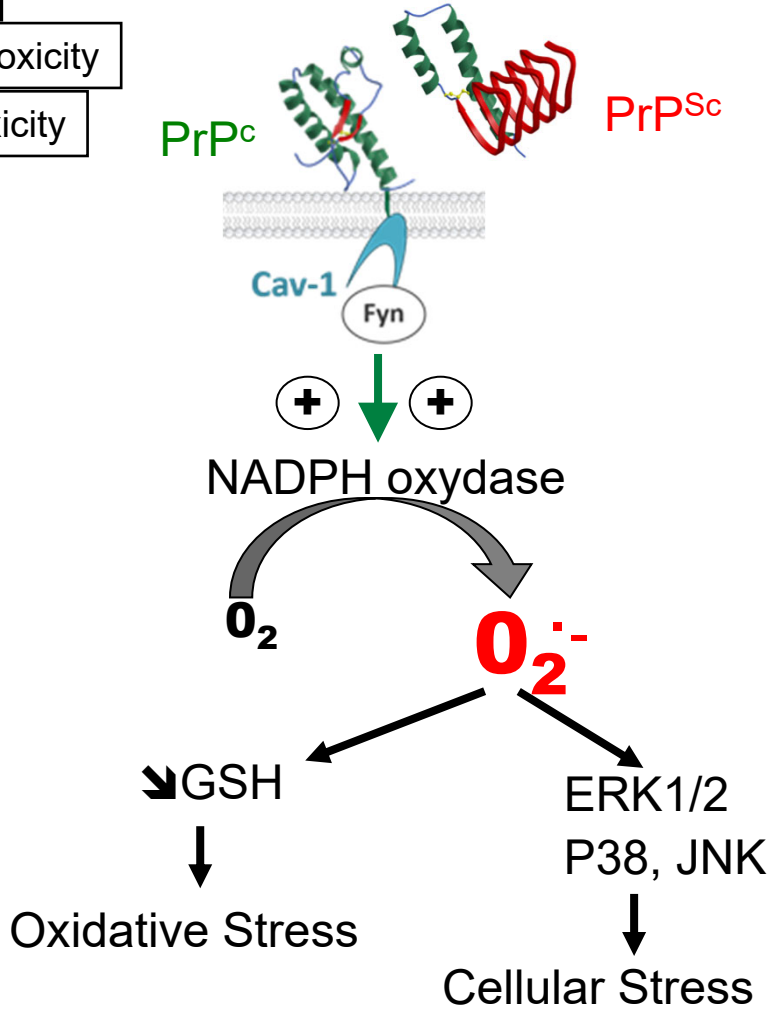
From signaling …



…to neurotoxicity



= Gain of function



Fine tuning of redox equilibrium

=> Neuronal homeostasis

Lost of neuronal homeostasis

=> Neuronal death

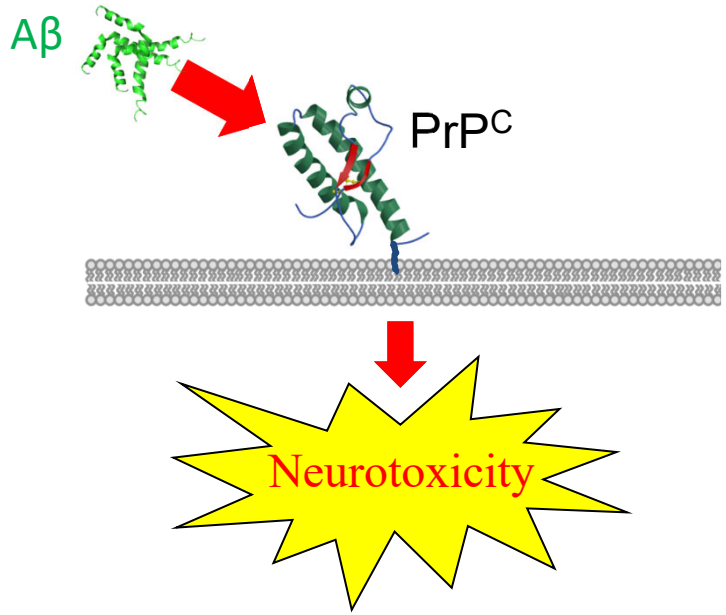
Prion disease and Alzheimer's disease: same fight?

nature Vol 457|26 February 2009|doi:10.1038/nature07761

LETTERS

Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers

Juha Laurén[1], David A. Gimbel[1], Haakon B. Nygaard[1], John W. Gilbert[1] & Stephen M. Strittmatter[1]



✓ Aβ peptides bind to PrP^C with high affinity.
✓PrP^C could relay neurotoxicity of Aβ peptides !
=> Mechanisms?

Alzheimer's disease
Discovery

- Auguste D, a 51-year-old woman : shown progressive cognitive impairment, focal symptoms, hallucinations, delusions, and psychosocial incompetence.

- At necropsy, there were plaques, neurofibrillary tangles, and arteriosclerotic changes.

Alois Alzheimer
(1864-1915)

Bielkhowsky's stain section from Auguste Deter's brain (left) neurofibrillary tangles drawn by Alois Alzheimer (right)

First description of tangle and plaque pathology
by Alois Alzheimer (1901)

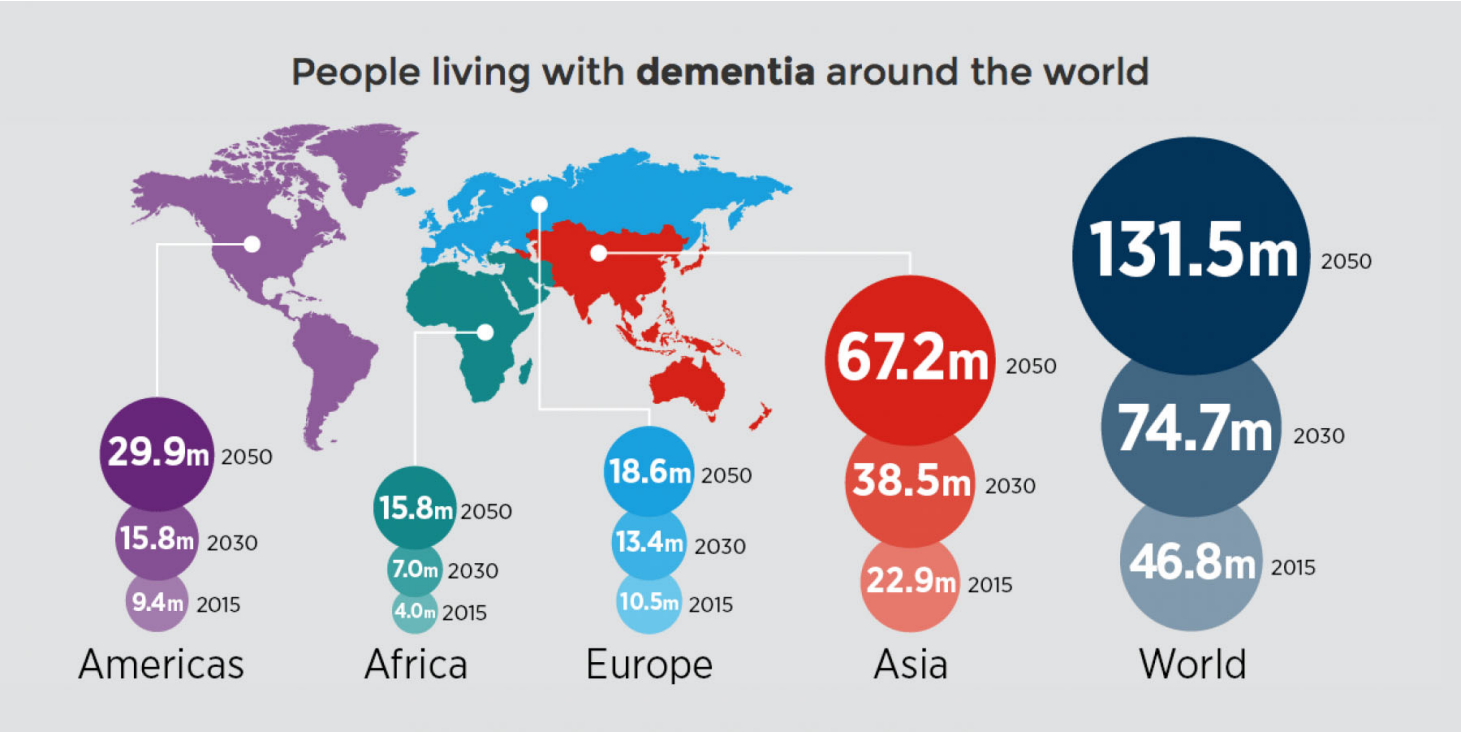
Alzheimer A. Über eine eigenartige Erkrankung der Hirnrinde. 1907 Jan ; 64:146-8.

Alzheimer's disease (AD)
Epidemiology

- ✓ AD is the most common form of dementia accounting for >60% of all the cases.

  In France: ~1M in 2019; +225 000 /year

  Word: 35.6M in 2019; +7.7M /year



World Alzheimer Report 2015

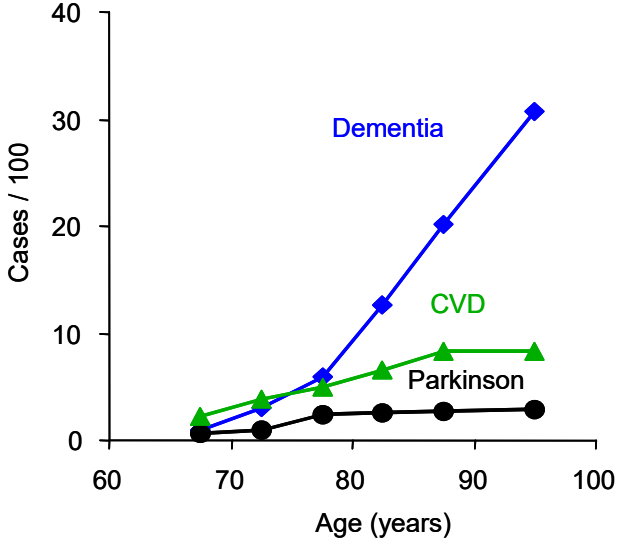
Alzheimer's disease (AD)
Epidemiology

✓ Most of the cases of AD *ARE SPORADIC*.

The prevalence of inherited forms of AD is <1%

✓ Risk factors for sporadic AD:

- aging
- head injuries
- hormonal changes
- vascular diseases
- inflammation
- ApoE e4 allele polymorphism
- exposure to metals (Al^{+++},Cu^{++} and Zn^{++})

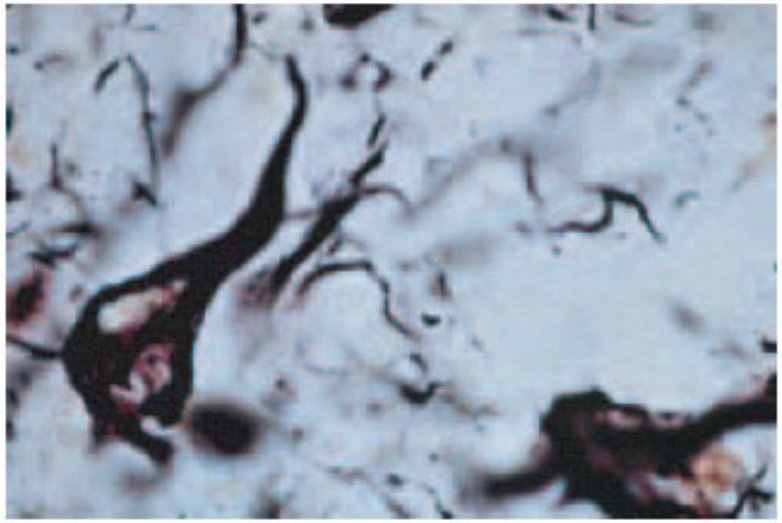


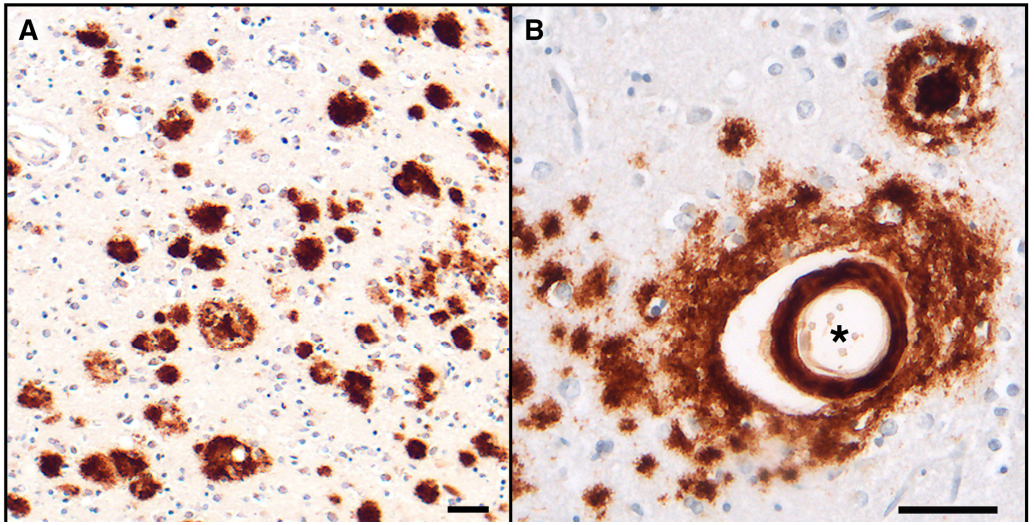
ApoE polymorphism and disease risk (alzdiscovery.org/)

Genotype	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4
Disease Risk	40% less likely	40% less likely	2.6 times more likely	Average risk	3.2 times more likely	14.9 times more likely

Alzheimer's disease (AD)
Histopathological hallmarks

❑ It is characterized by the presence of lesions both at an extracellular level (the β-amyloid plaques), and at an intracellular levels (the Neurofibrillary tangles, NFT).





Neurofibrillary tangles

➢ hyperphosphorylated protein tau

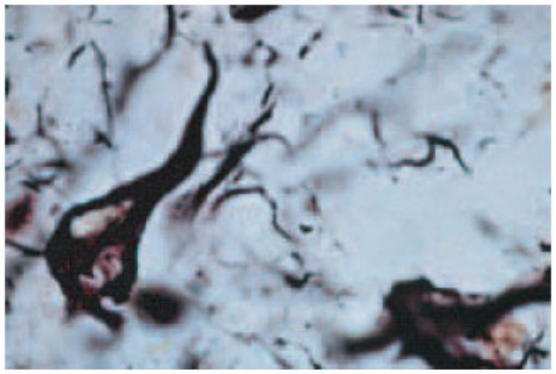
Amyloid plaques (A) and Cerebral β-Amyloid Angiopathy (CAA, B *)

➢protein β-amyloid (Aβ) fibrils

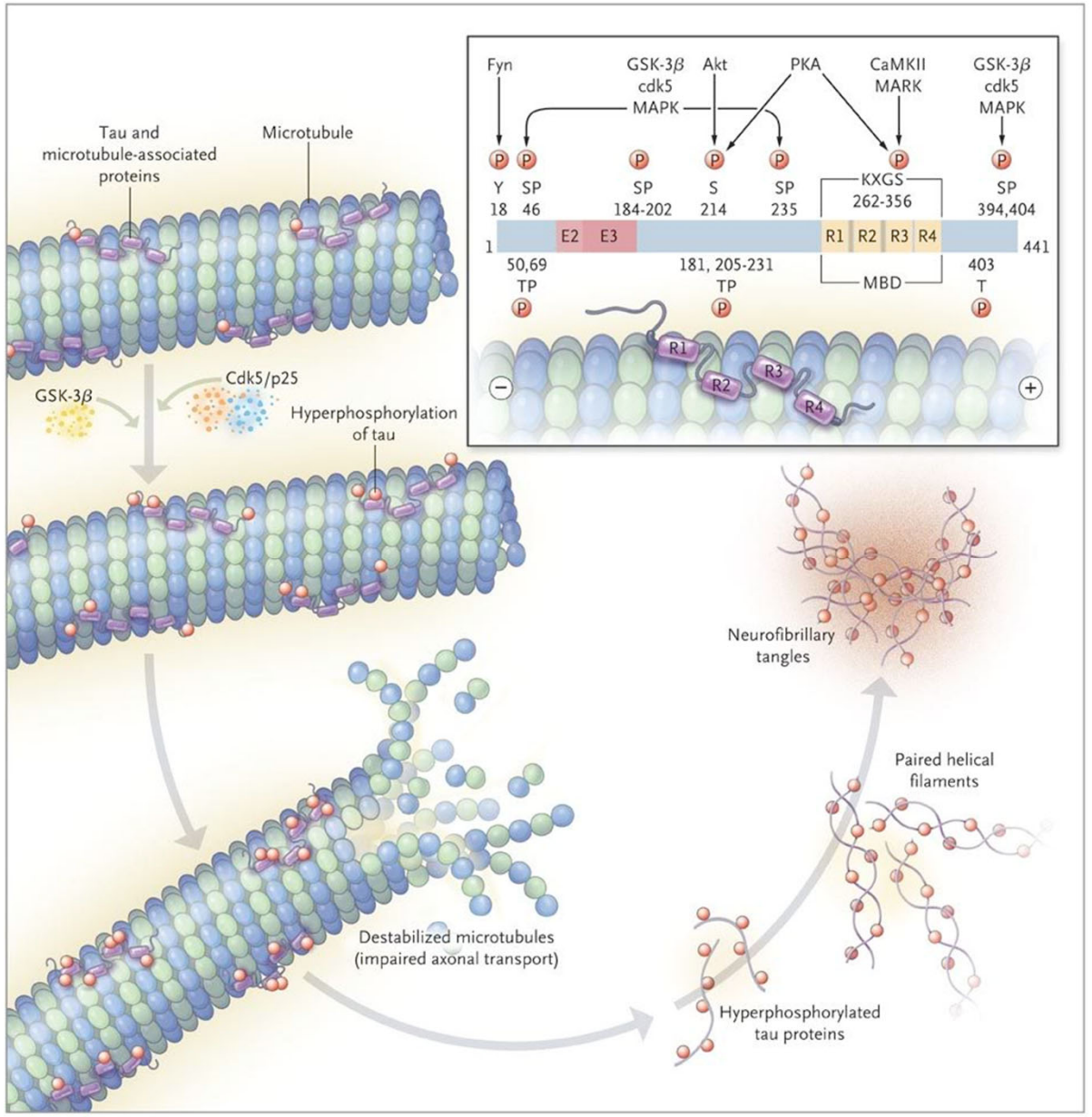
M. Jucker, et al. Alzheimer's disease: From immunotherapy to immunoprevention, Cell, 2023

Alzheimer's disease (AD)
Formation of NFTs

(a)



Tau based neurofibrillary tangle



Alzheimer's disease (AD)
β-amyloid Plaque

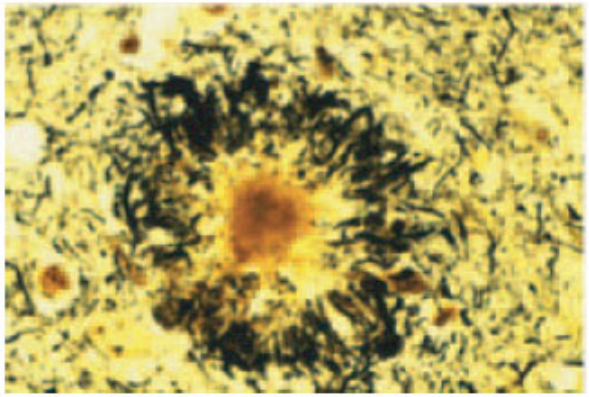
-is extracellular

-normally, it has a core composed of the β-amyloid peptide

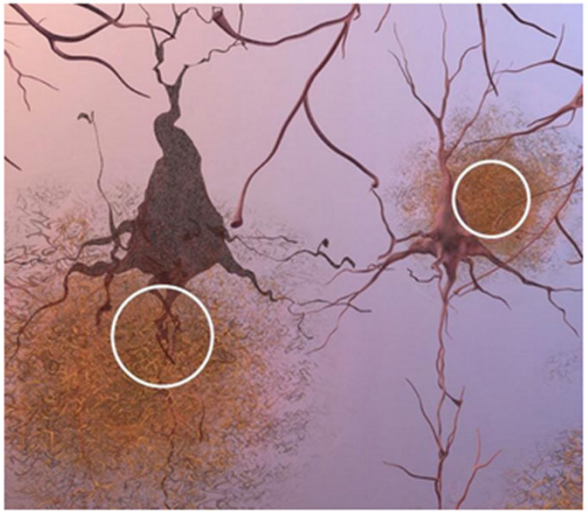
-aggregation is massive in the center, and diffused at the sides (disaggregation hypothesis?)

-average aera 400 μm^2

-is composed of dystrophic neurites, β-amyloid peptides (40/42/43), ubiquitin, tau protein and other proteins, some involved in the generation of β-amyloid, like the secretases.

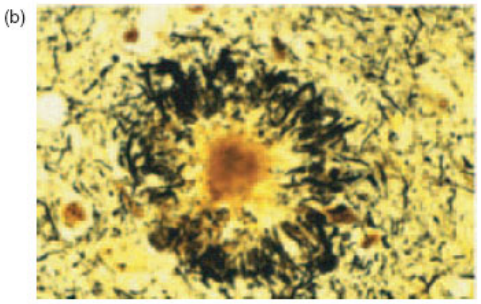


Amyloid based Neuritic plaque

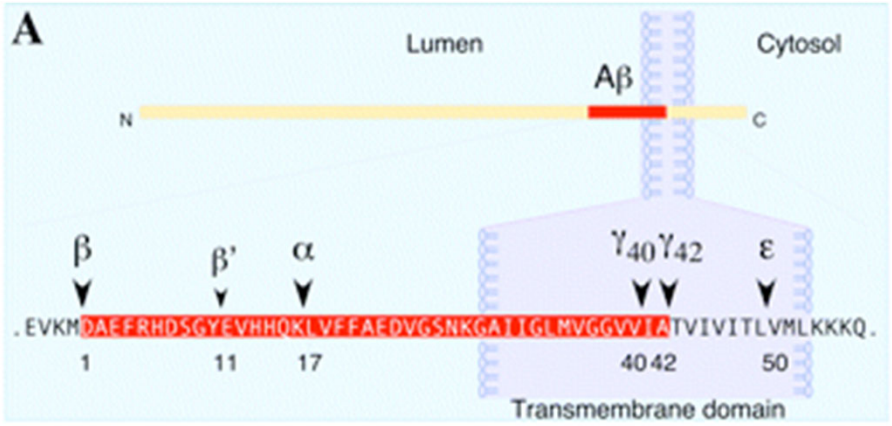


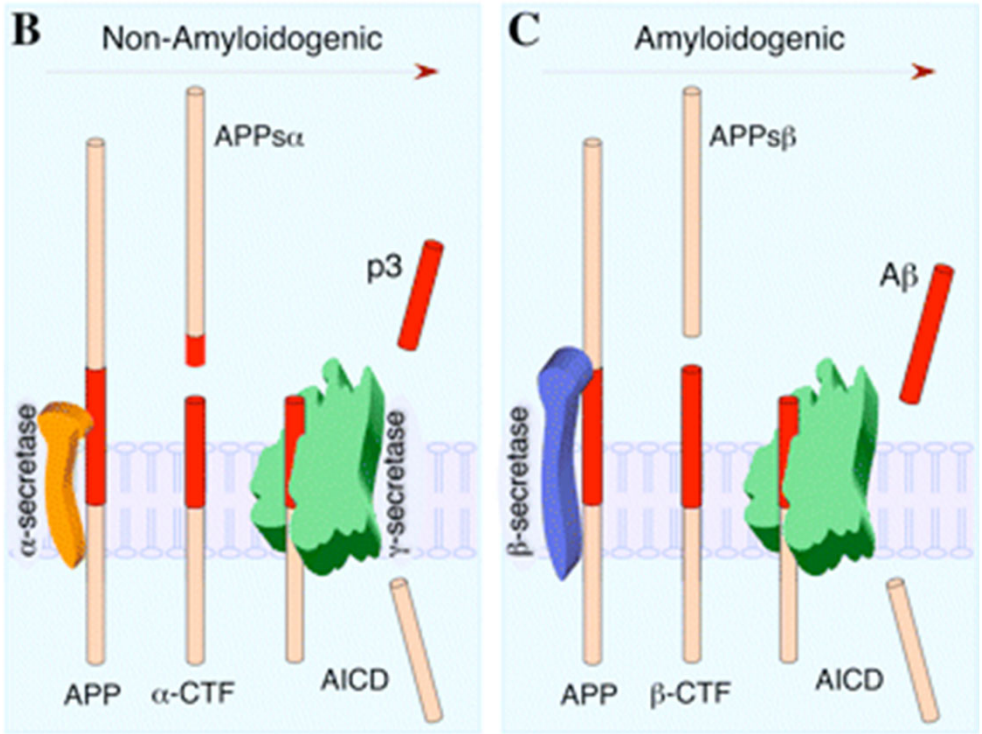
Plaques, abnormal clusters of protein fragments, build up between nerve cells.

Alzheimer's disease (AD)
Origin of β-amyloid plaques: APP processing



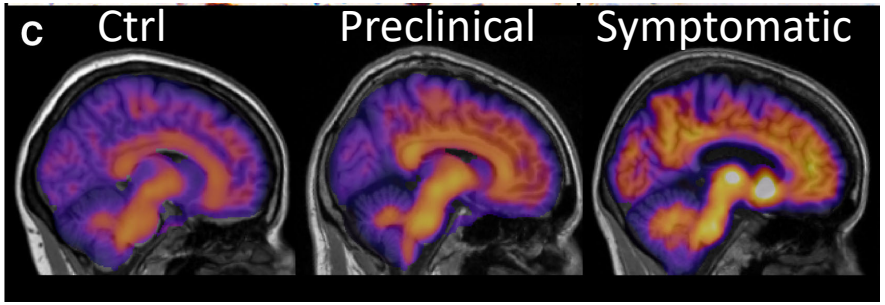
Amyloid based Neuritic plaque

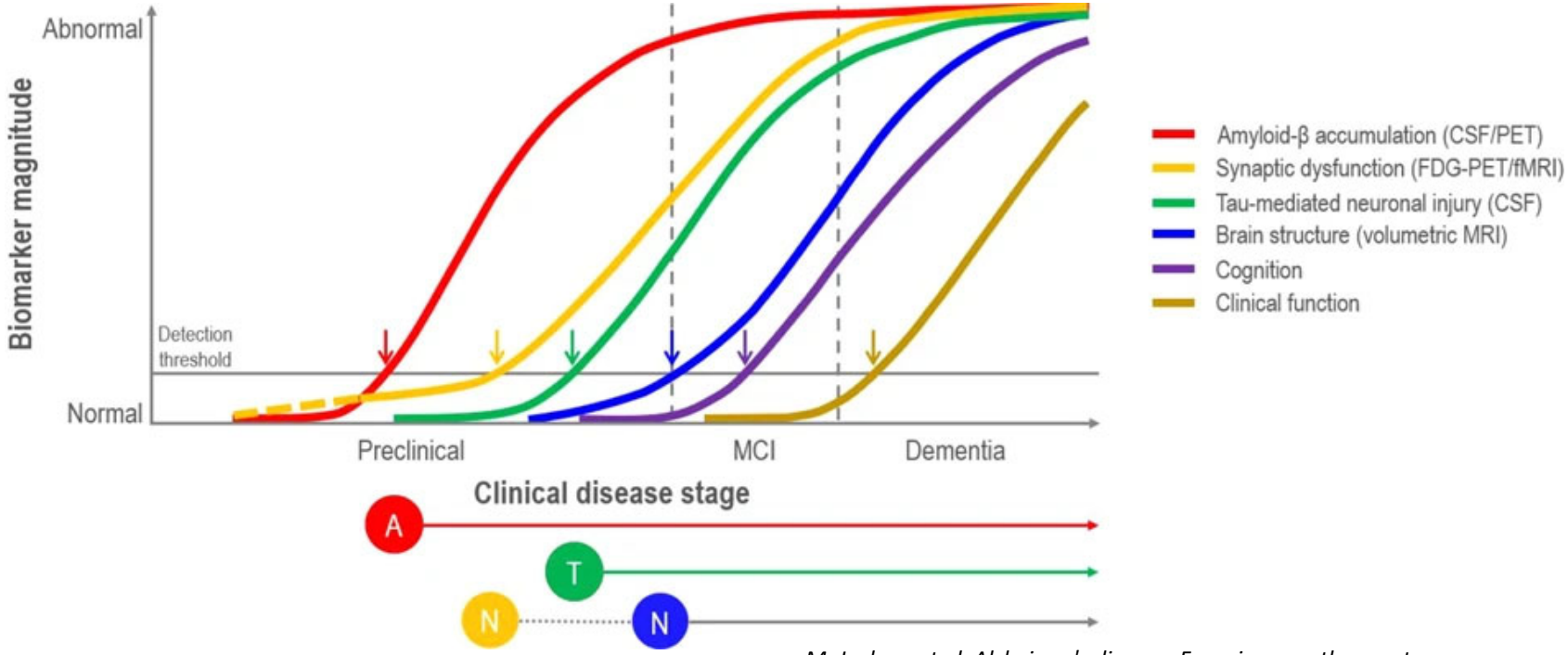




Alzheimer’s disease (AD)
Dynamic biomarkers

Aβ-PET images with Pittsburgh compound tracer in familial AD

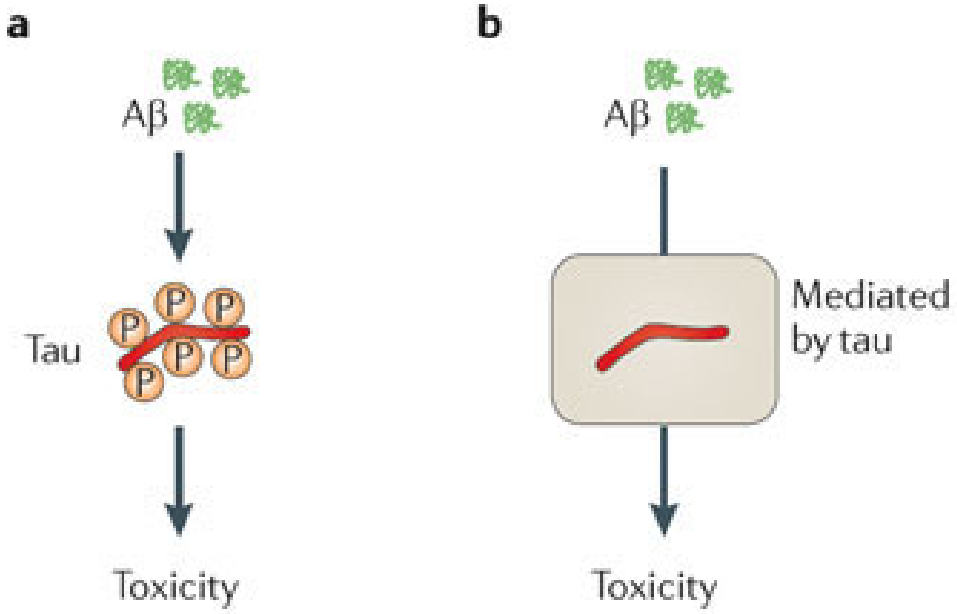




M. Jucker, et al. Alzheimer's disease: From immunotherapy to immunoprevention, Cell, 2023

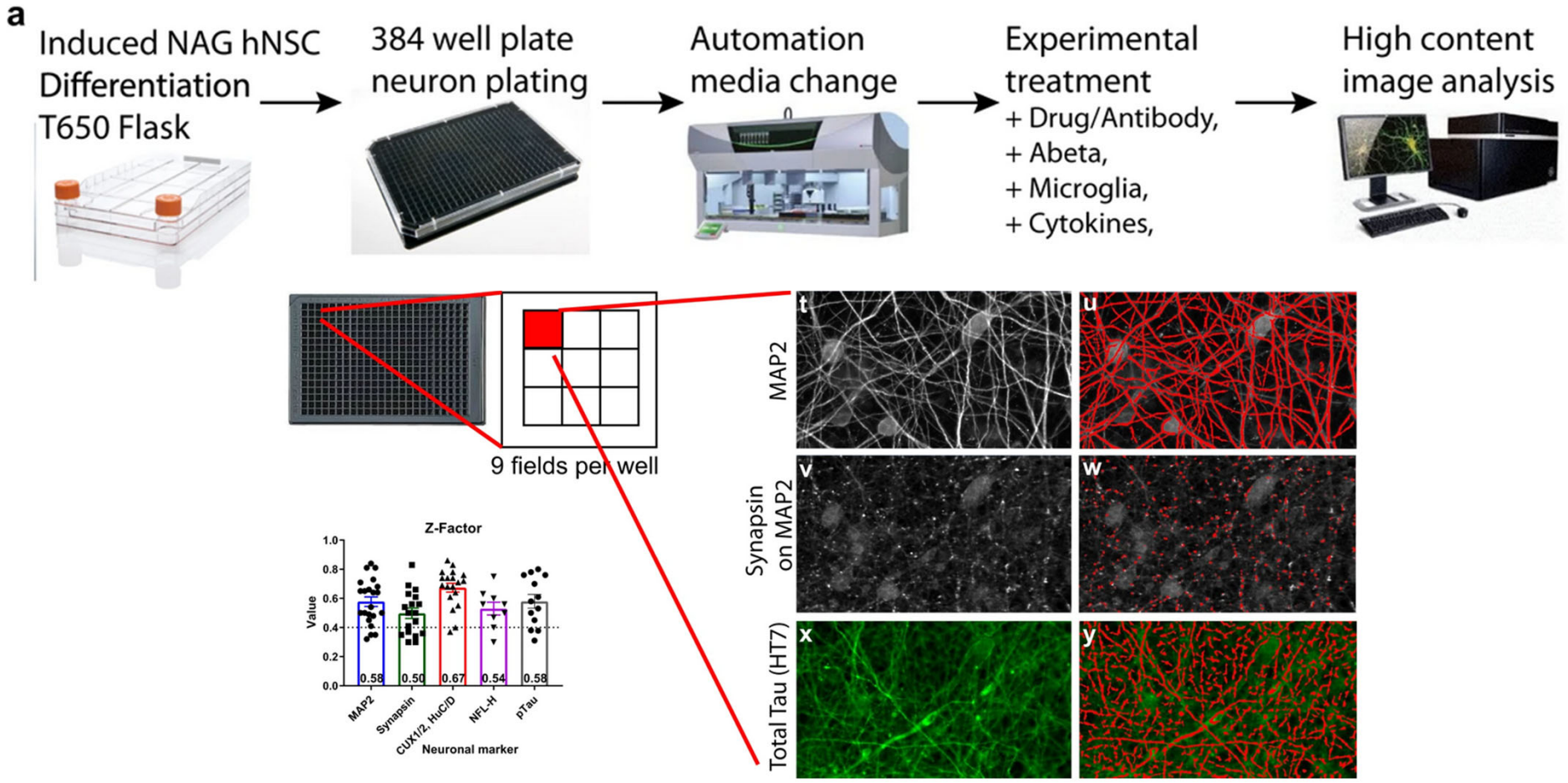
Alzheimer's disease (AD)
β-amyloid peptide/Tau toxicities

- Amyloid-β and tau: interaction for toxicity



Alzheimer's disease (AD)
β-amyloid peptide/Tau toxicities

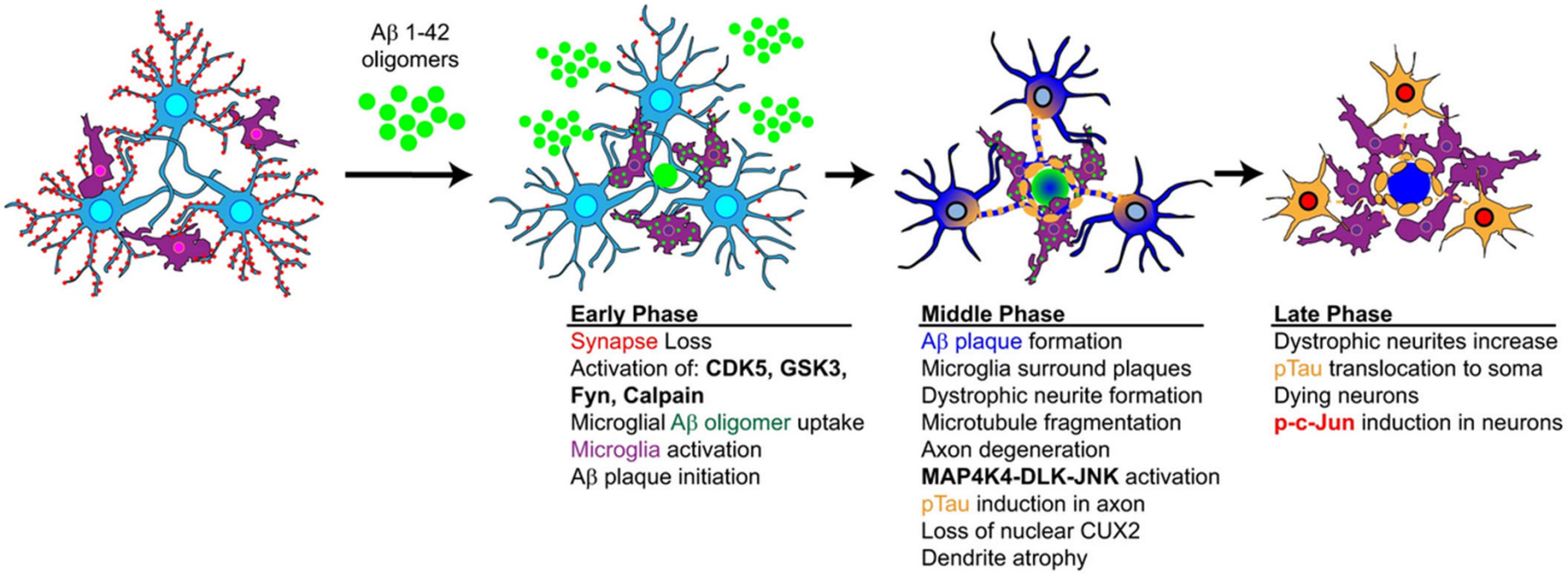
A high-throughput, automated human iPSC-derived neuron differentiation and culturing platform = a new model of AD



Mature culture (12 weeks) => image analysis

Alzheimer's disease (AD)
β-amyloid peptide/Tau toxicities

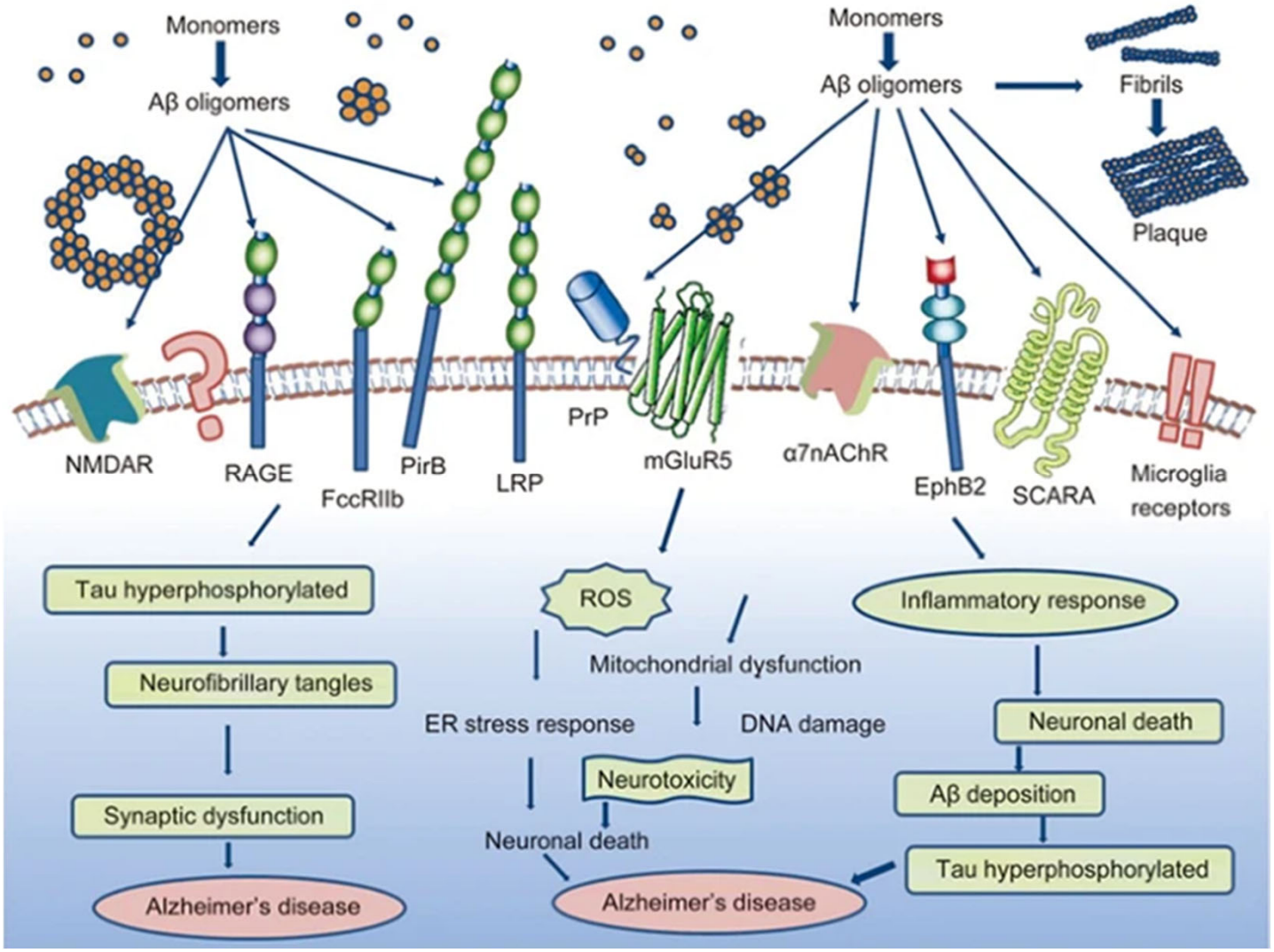
❑ Amyloid-β and tau: interaction for toxicity
=> Role of microglia



Aβ and microglial activation as two partially independent processes that, when they converge, lead to neocortical tau pathology

Bassil, R., Shields, K., Granger, K. et al. Improved modeling of human AD with an automated culturing platform for iPSC neurons, astrocytes and microglia. Nat Commun ***12,*** *5220 (2021)*

Alzheimer's disease (AD)
Amyloid cascade hypothesis



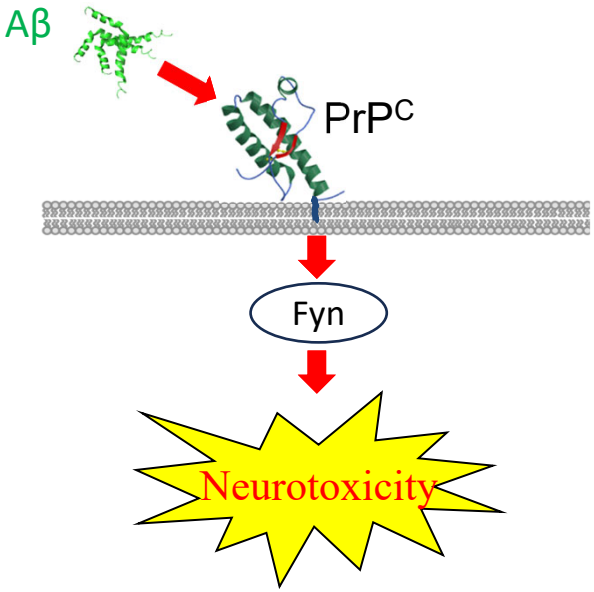
Do Prion and Alzheimer's diseases shared common neurodegenerative mechanisms?

Alzheimer amyloid-β oligomer bound to postsynaptic prion protein activates Fyn to impair neurons

Ji Won Um[1], Haakon B Nygaard[1], Jacqueline K Heiss[1], Mikhail A Kostylev[1], Massimiliano Stagi[1], Alexander Vortmeyer[2], Thomas Wisniewski[3], Erik C Gunther[1] & Stephen M Strittmatter[1]

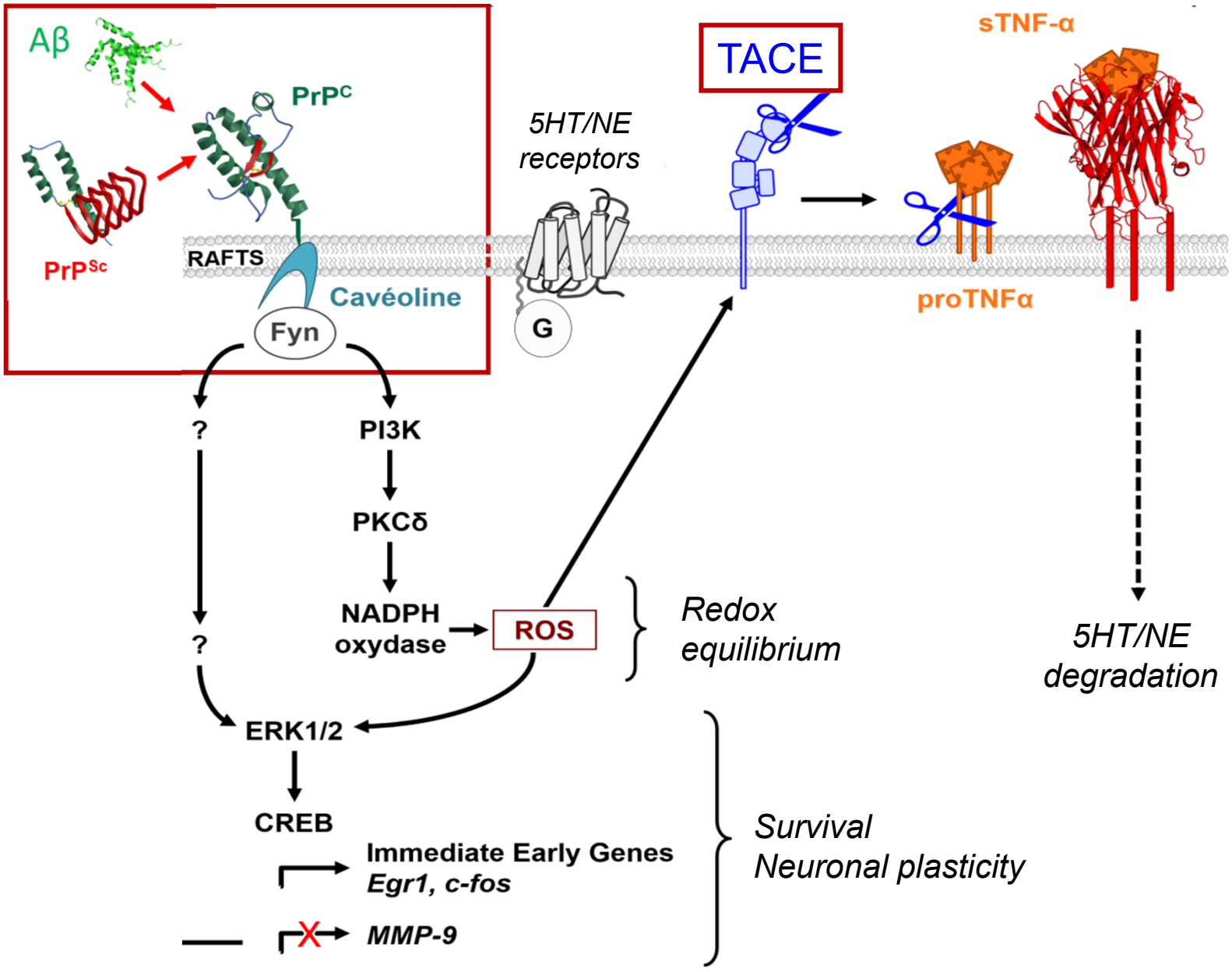
Amyloid-beta (Aβ) oligomers are thought to trigger Alzheimer's disease pathophysiology. Cellular prion protein (PrP^C) selectively binds oligomeric Aβ and can mediate Alzheimer's disease–related phenotypes. We examined the specificity, distribution and signaling of Aβ-PrP^C complexes, seeking to understand how they might alter the function of NMDA receptors (NMDARs) in neurons. PrP^C is enriched in postsynaptic densities, and Aβ-PrP^C interaction leads to Fyn kinase activation. Soluble Aβ assemblies derived from the brains of individuals with Alzheimer's disease interacted with PrP^C to activate Fyn. Aβ engagement of PrP^C-Fyn signaling yielded phosphorylation of the NR2B subunit of NMDARs, which was coupled to an initial increase and then a loss of surface NMDARs. Aβ-induced dendritic spine loss and lactate dehydrogenase release required both PrP^C and Fyn, and human familial Alzheimer's disease transgene–induced convulsive seizures did not occur in mice lacking PrP^C. These results delineate an Aβ oligomer signal transduction pathway that requires PrP^C and Fyn to alter synaptic function, with deleterious consequences in Alzheimer's disease.

nature neuroscience



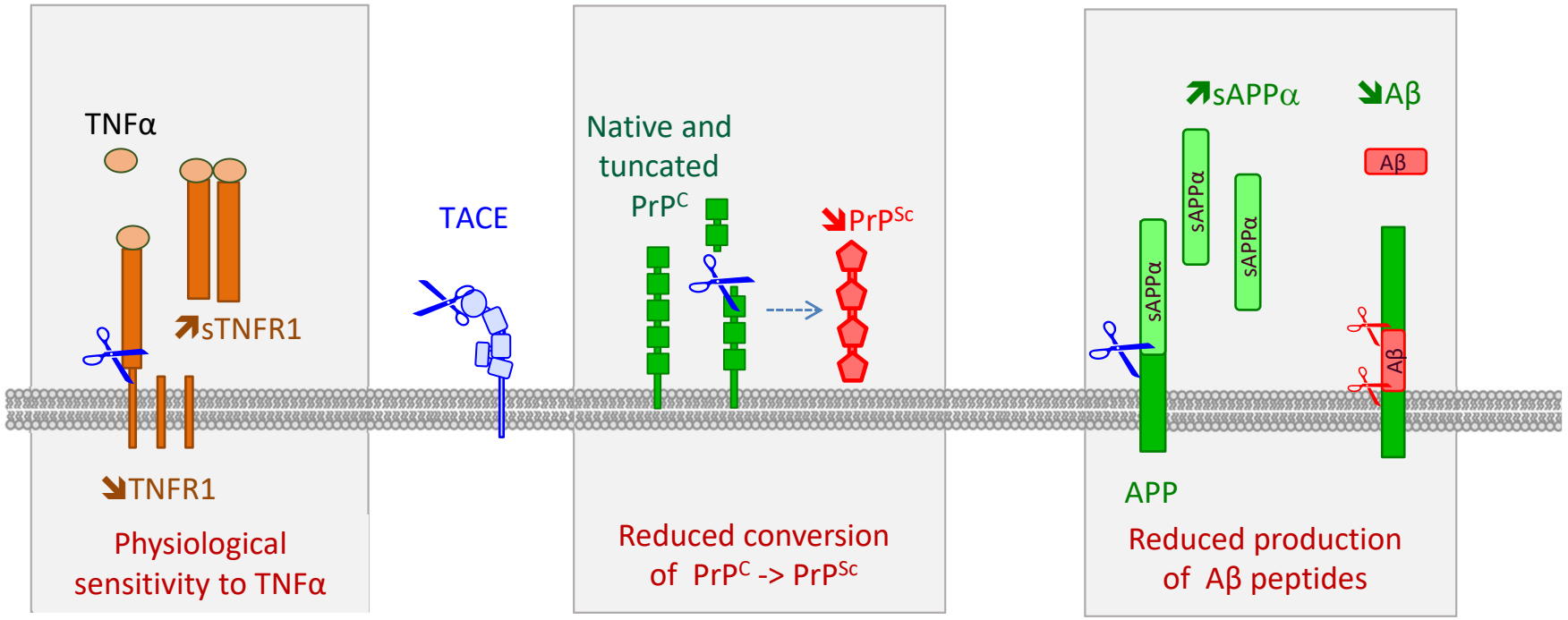
Nature neuroscience, 2012

Do Prion and Alzheimer's diseases shared common neurodegenerative mechanisms?



TACE physiological functions

Nature Medicine, 2013



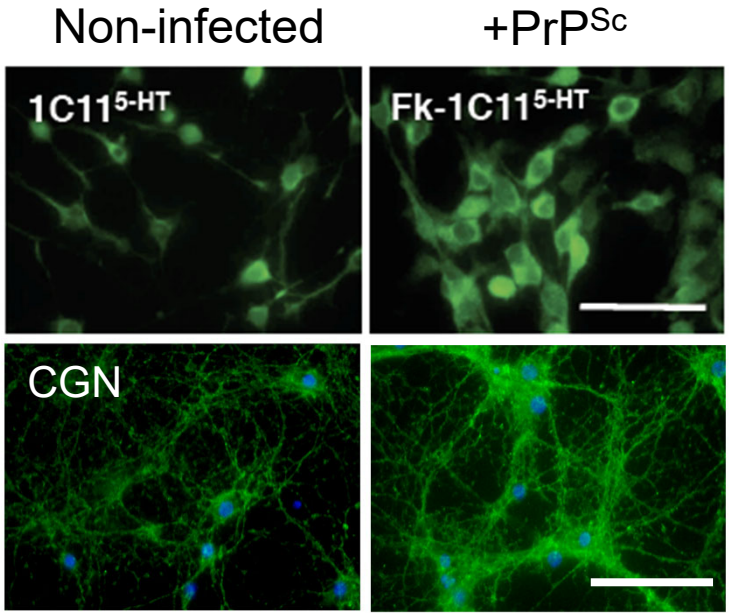
TACE deregulation => prion / Alzheimer's diseases ?

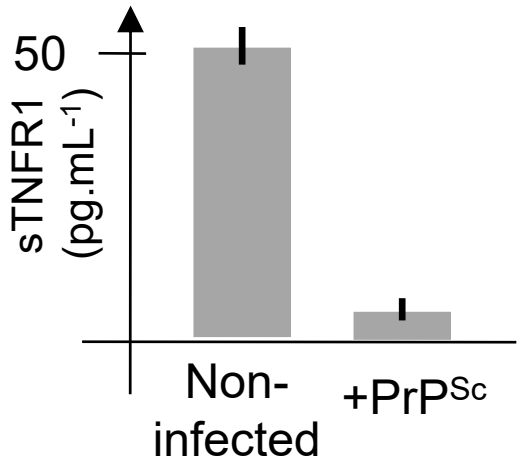
Little was known about TACE regulation

Prion infection triggers TNFR1 under-shedding and hypersensitizes cells to TNFα toxicity

↗ cell surface level of TNFR1

↘ sTNFR1 in cell culture medium





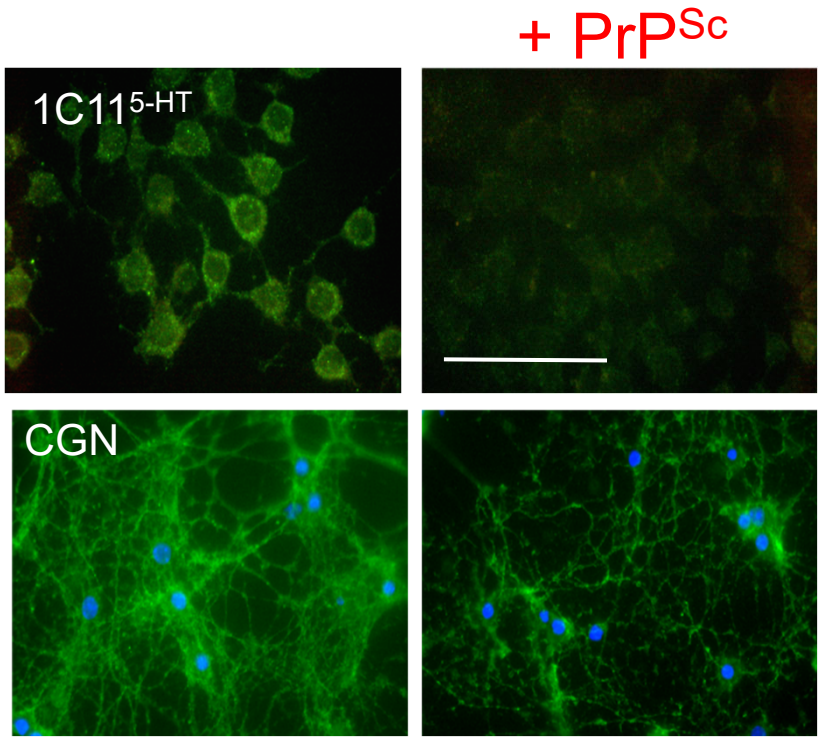
CGN = Cerebellar Granule Neuron

↗ sensitivity of infected neurons to TNFα

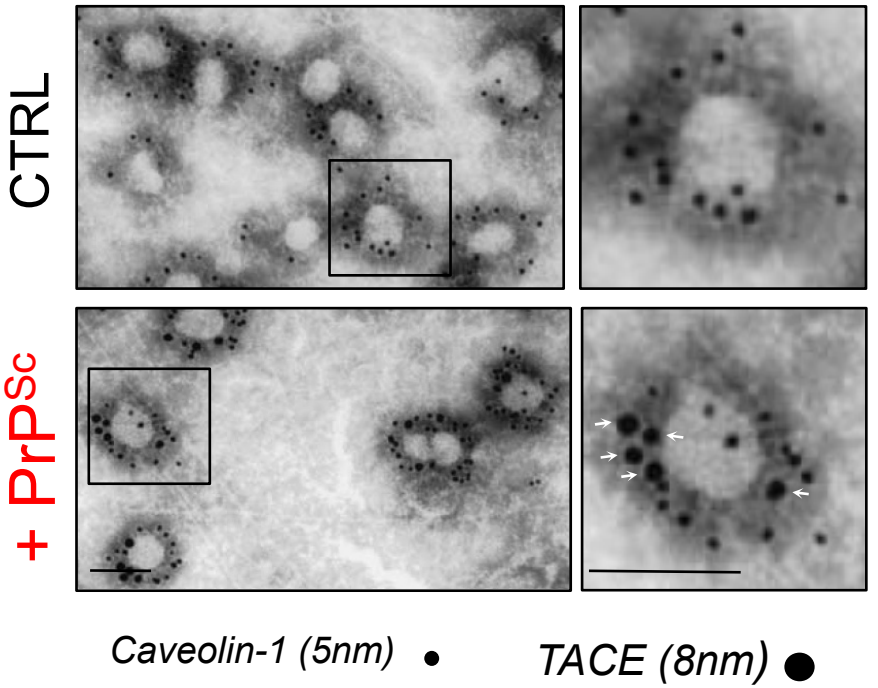
Defect of TACE shedding activity in prion-infected neurons ?

TACE internalization in prion-infected cells

↘ TACE at the plasma membrane

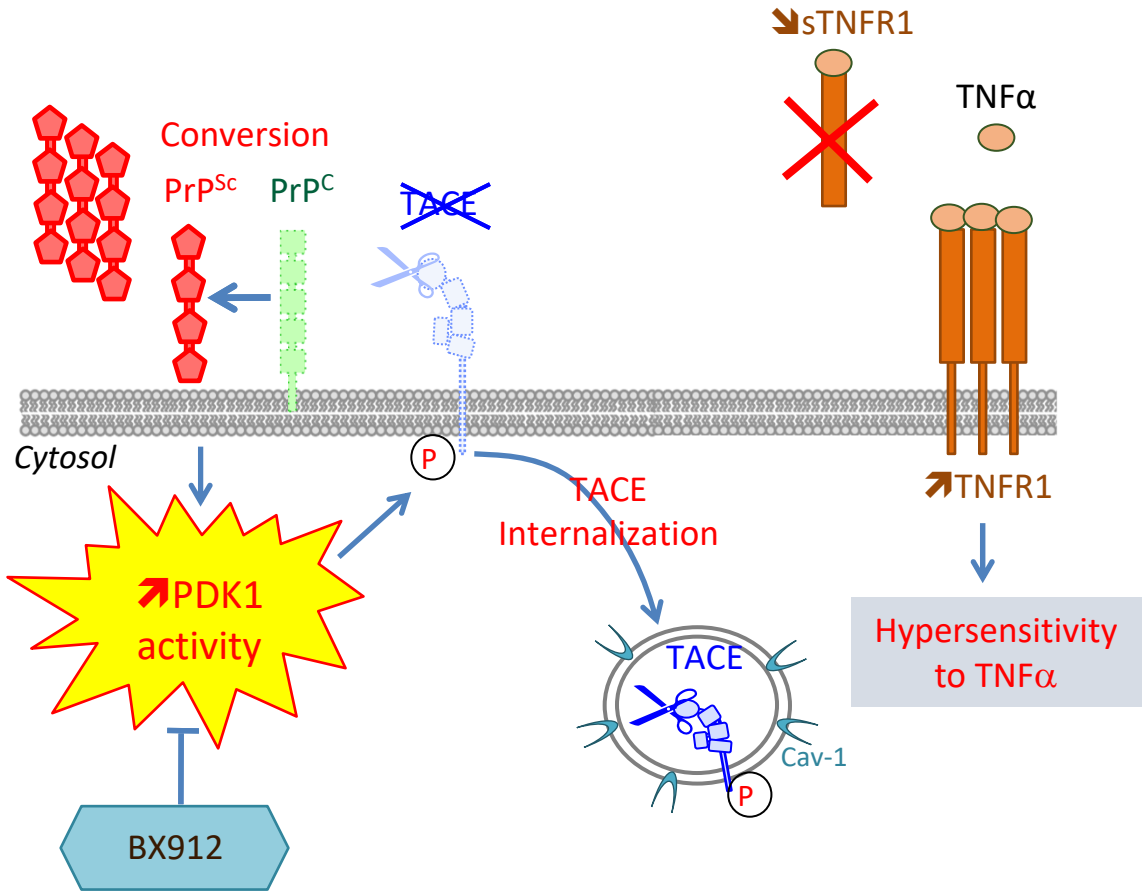


TACE is associated with caveolae in infected cells



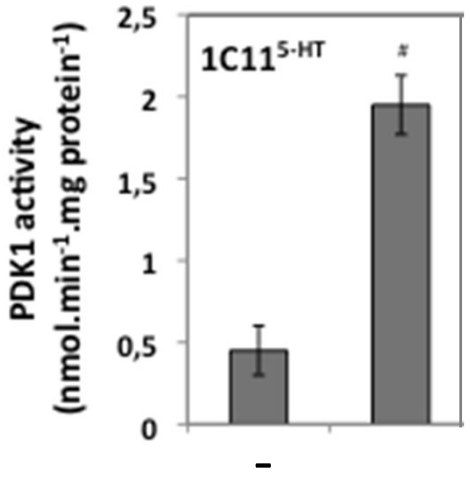
Coll J.M. Launay/ Hoffmann LaRoche

TACE internalization depends on PDK1 overactivity in prion-infected cells

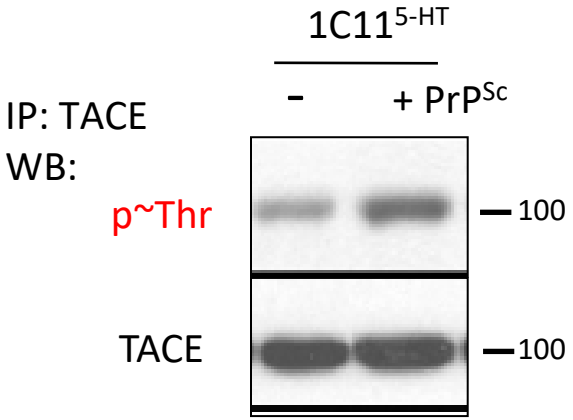


Antagonizing PDK1 activity to rescue TACE shedding activity at the plasma membrane?

PDK1 activity



phospho-TACE

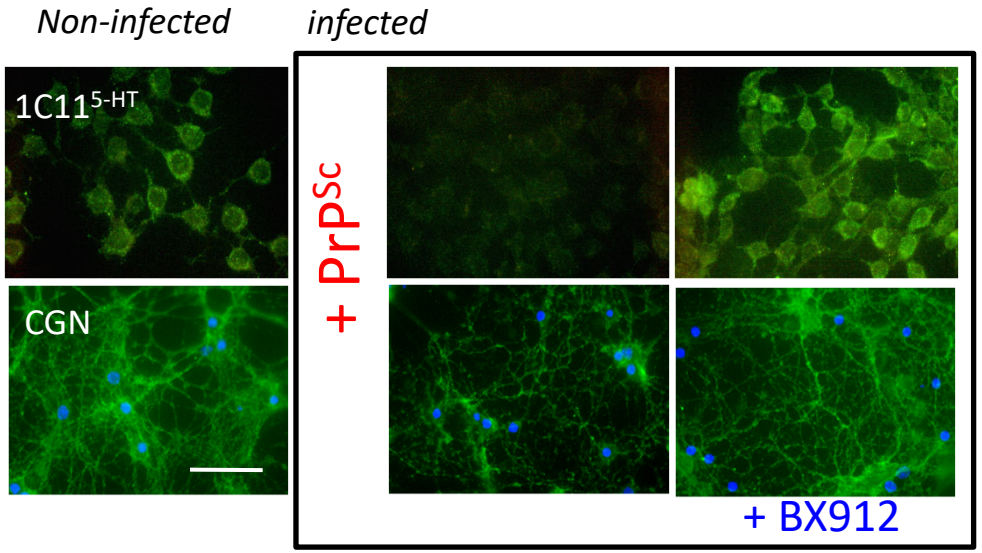


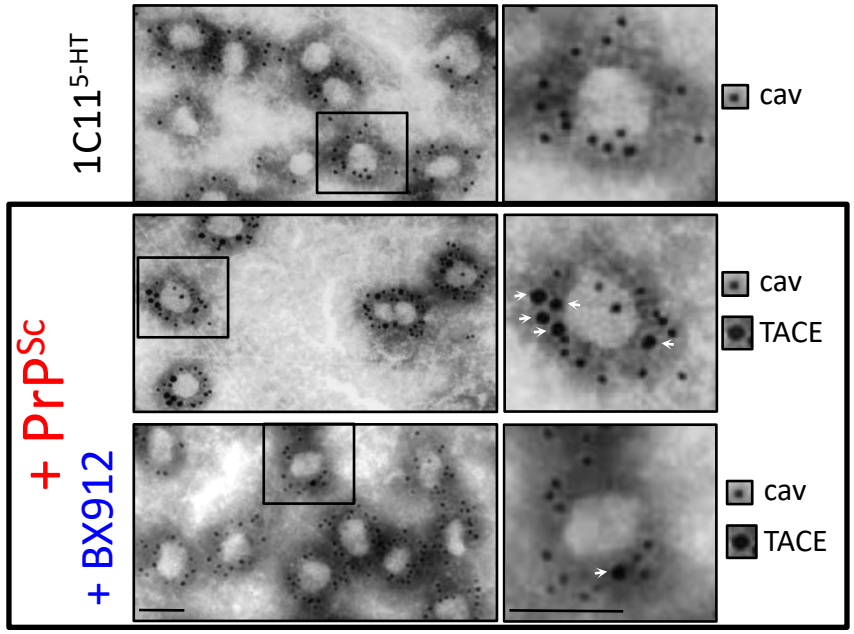
PDK1 = 3-phosphoinositide-dependent kinase 1

Nature Medicine, 2013

PDK1 inhibition relocates TACE from caveolae to the plasma membrane

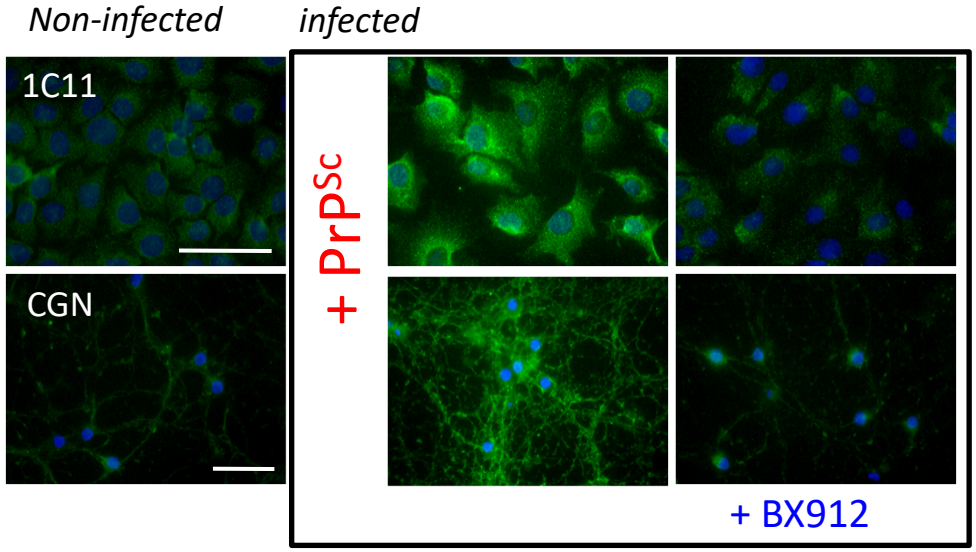
Cell surface Immunostaining of TACE



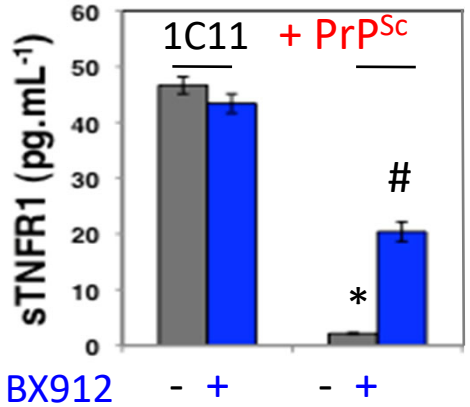


PDK1 inhibition restores TACE shedding activity at the plasma membrane

TNFR1



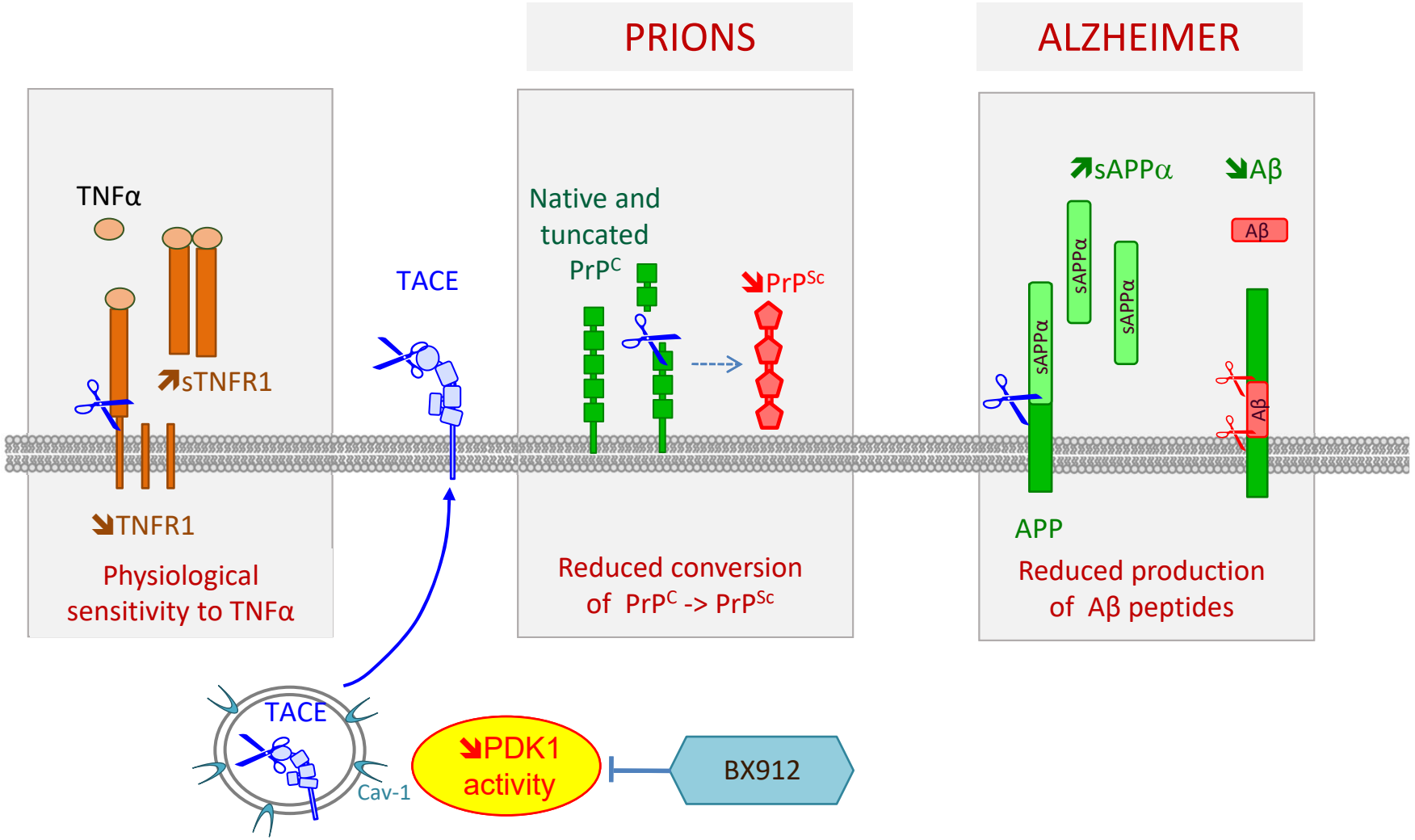
sTNFR1



BX912 rescues the shedding of TNFR1 by TACE

=> Desensitization of prion-infected cells from TNFα toxicity

PDK1 as a therapeutic target against prion and Alzheimer's diseases?

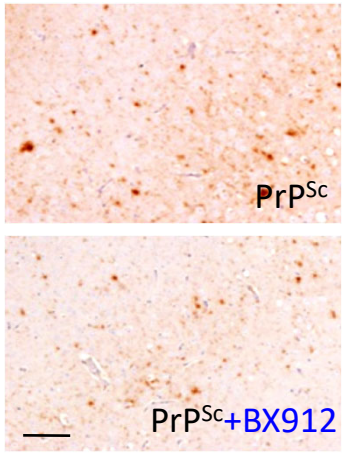


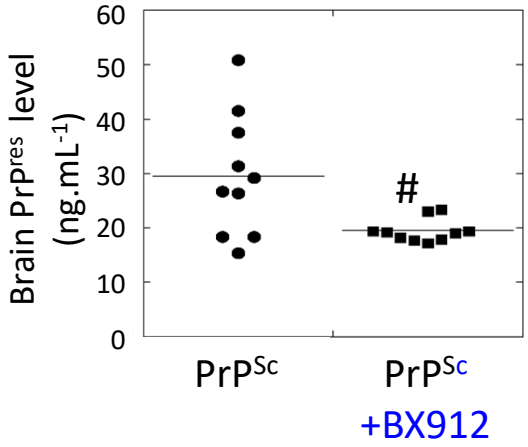
Thus relocated TACE would have 3 beneficial effects in neurodegenerative diseases

Inhibiting PDK1 activity with BX912 in prion-infected mice…

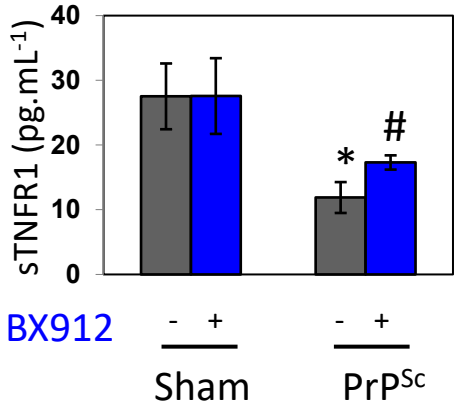
↘ brain PrP^{Sc}

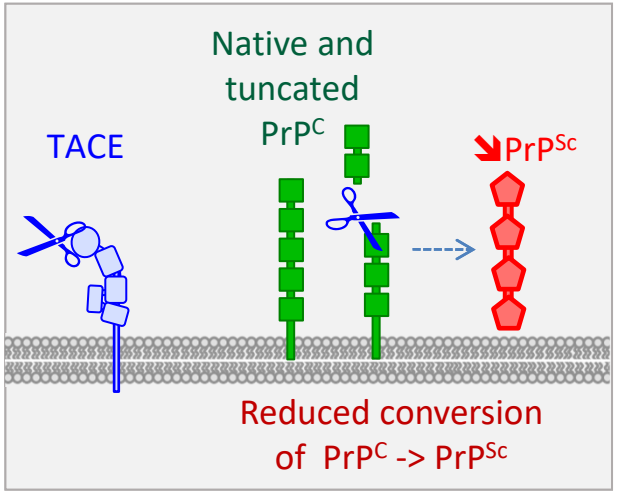
PrP^{Sc} deposition



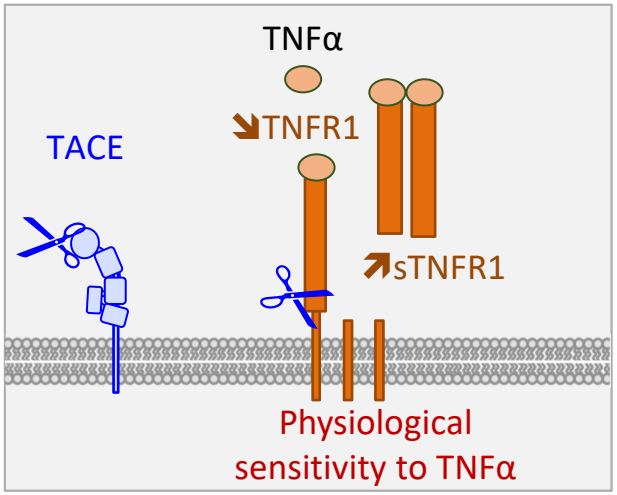


↗ sTNFR1 in CSF





Same effect upon PDK1 silencing with siRNA



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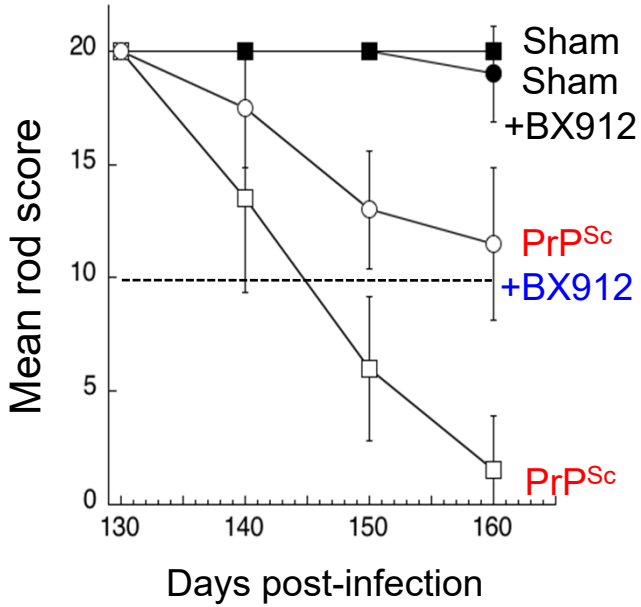
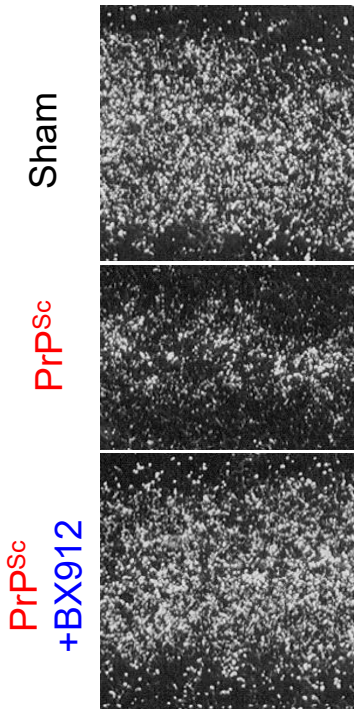
Inhibiting PDK1 activity with BX912 in prion-infected mice…

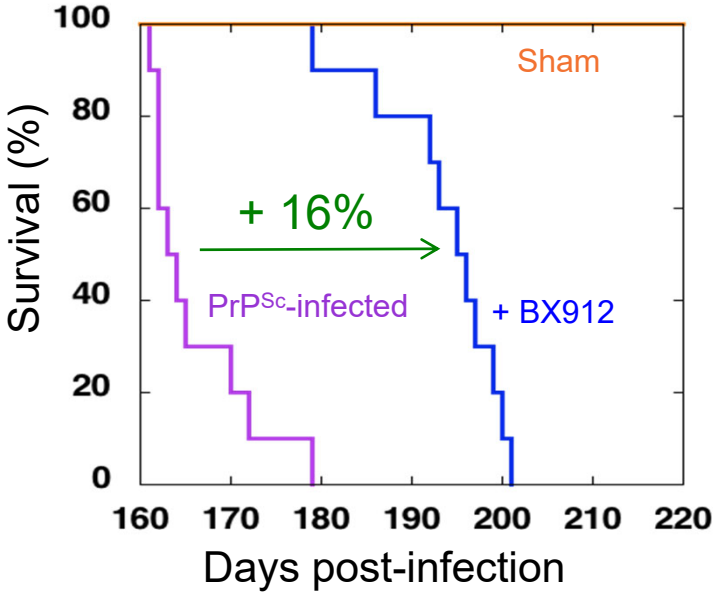
↘ motor deficits

↘ neurodegeneration in the IGL

↗ survival time



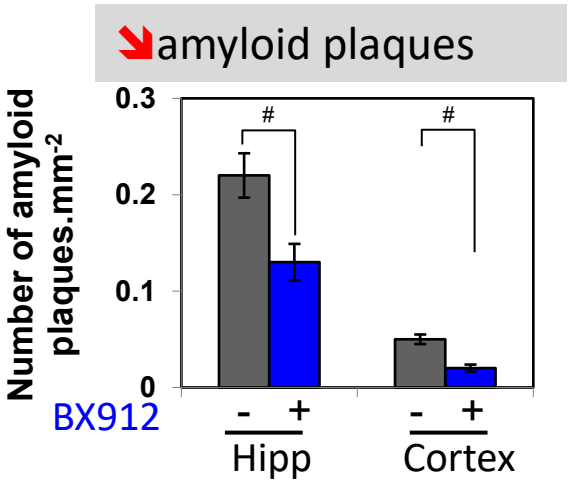


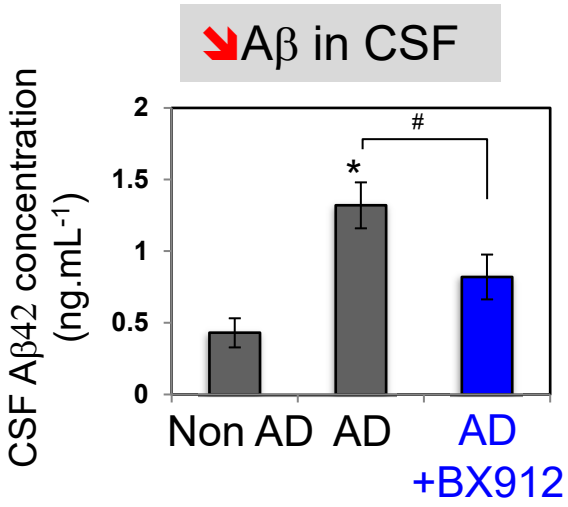


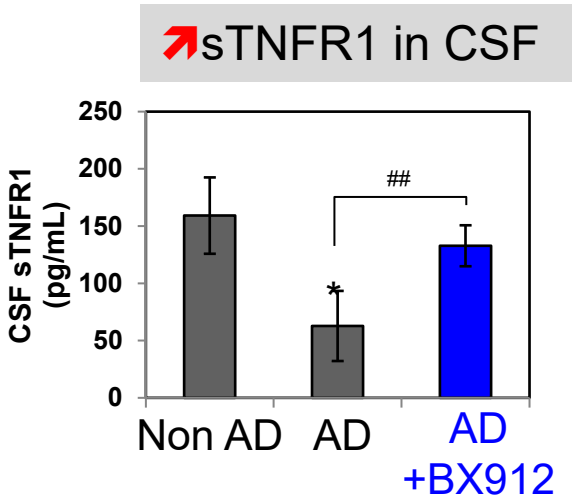
Coll. J.M. Launay/ Hoffmann LaRoche

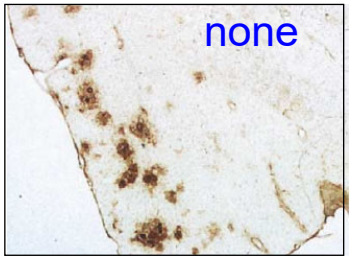
One drug for both prion and Alzheimer’s diseases ?

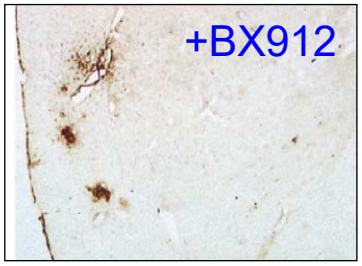
Inhibiting PDK1 activity with BX912 in Alzheimer's mouse models (Tg2576, 3xTg-AD, 5xTg-AD)…



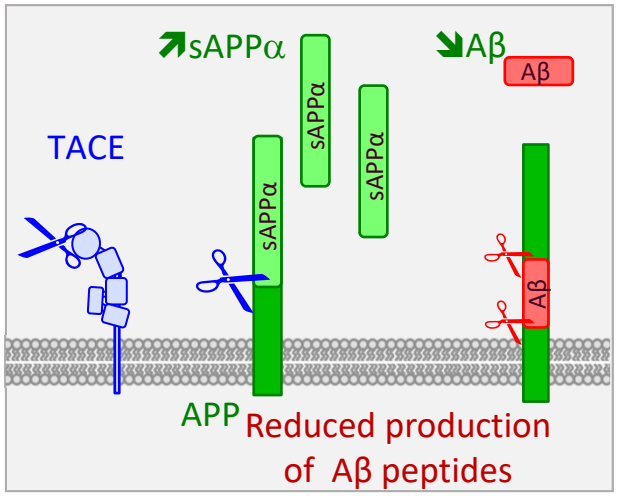








Cortex slices
Thioflavin S staining of amyloid plaques



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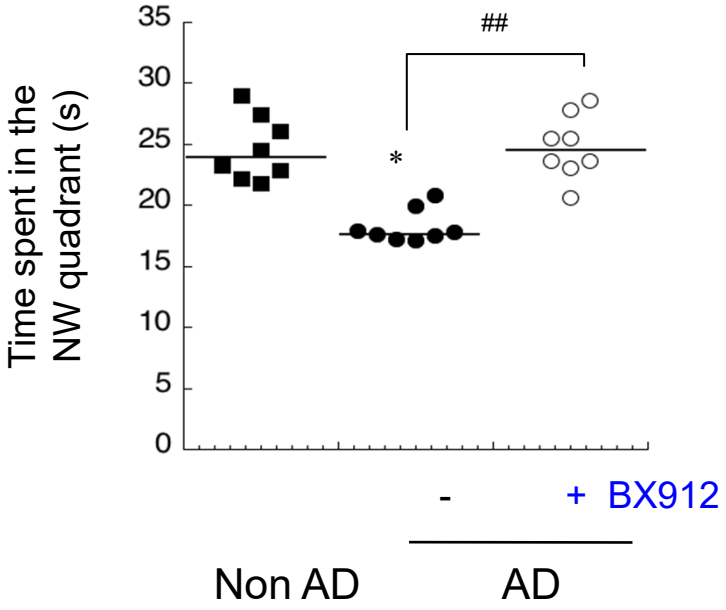
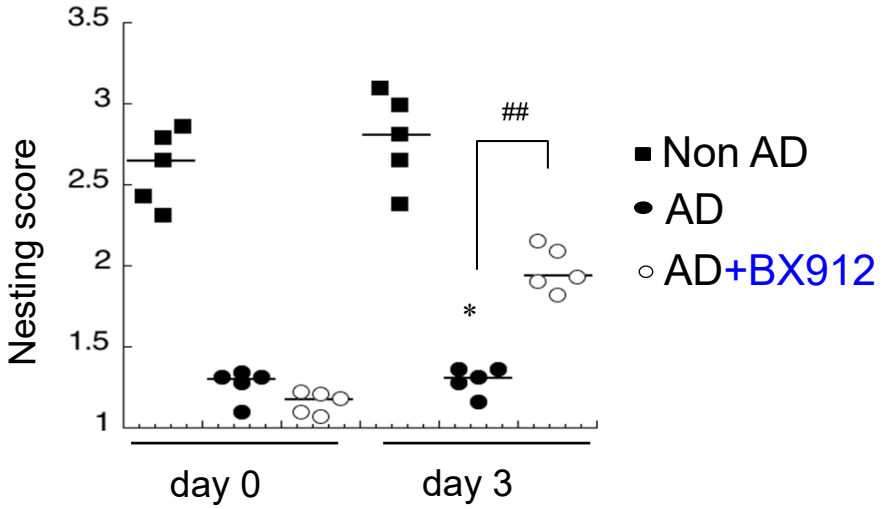
Inhibiting PDK1 activity with BX912 in Alzheimer's mouse models (Tg2576, 3xTg-AD, 5xTg-AD)…

↘ memory and cognitive impairments

Morris Water Maze



Nesting



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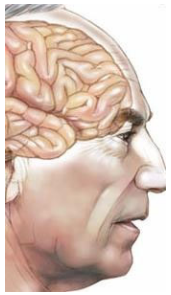
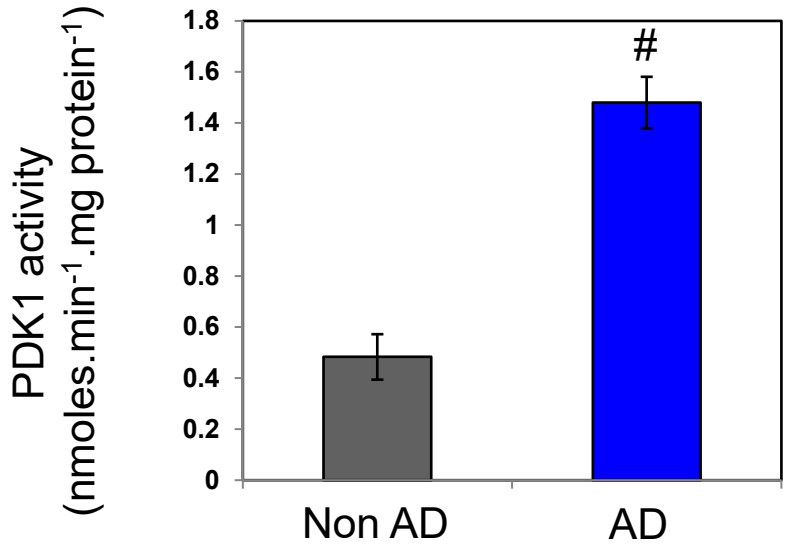
➔PDK1 inhibition alleviates both prion and Alzheimer's diseases in mice



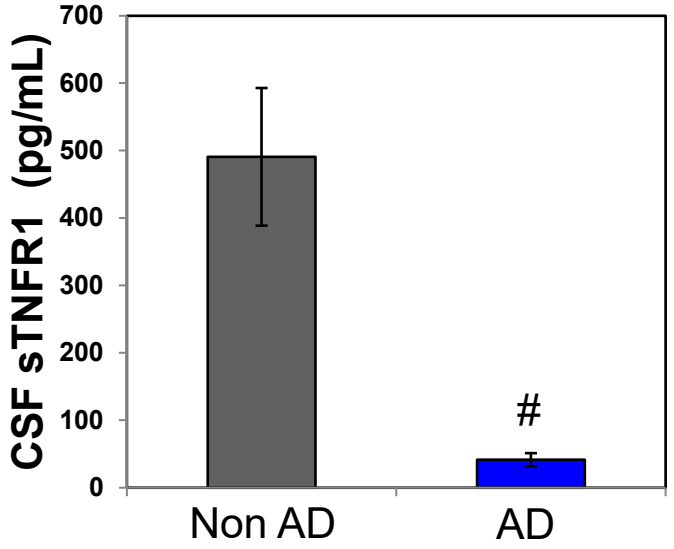
PDK1: a therapeutic target for AD patients ?

6 AD patients

↗ PDK1 activity in human AD brain



↘ sTNFR1 in CSF of AD patients



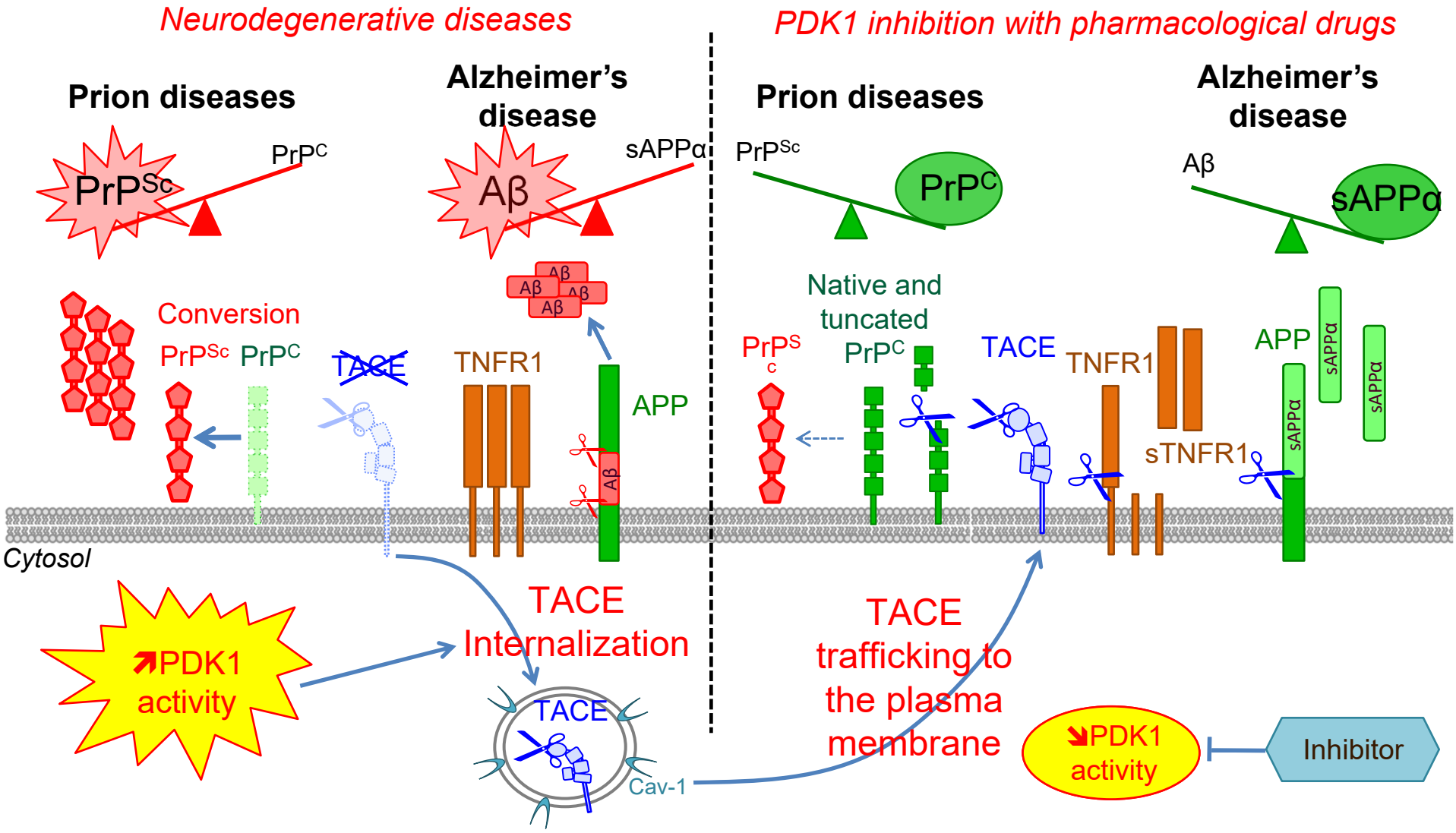
TACE internalization

Defect of TACE shedding activity
(Readout sTNFR1)

Coll. J.M. Launay/ Hoffmann LaRoche

Summary

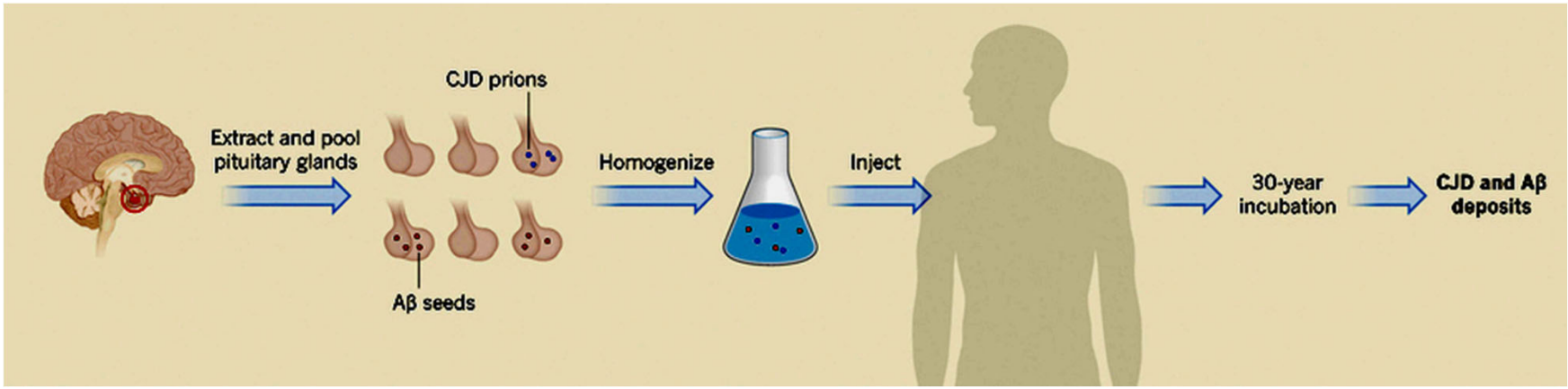
Nature Medicine, 2013



Prion and Alzheimer's diseases share common neurodegenerative mechanisms

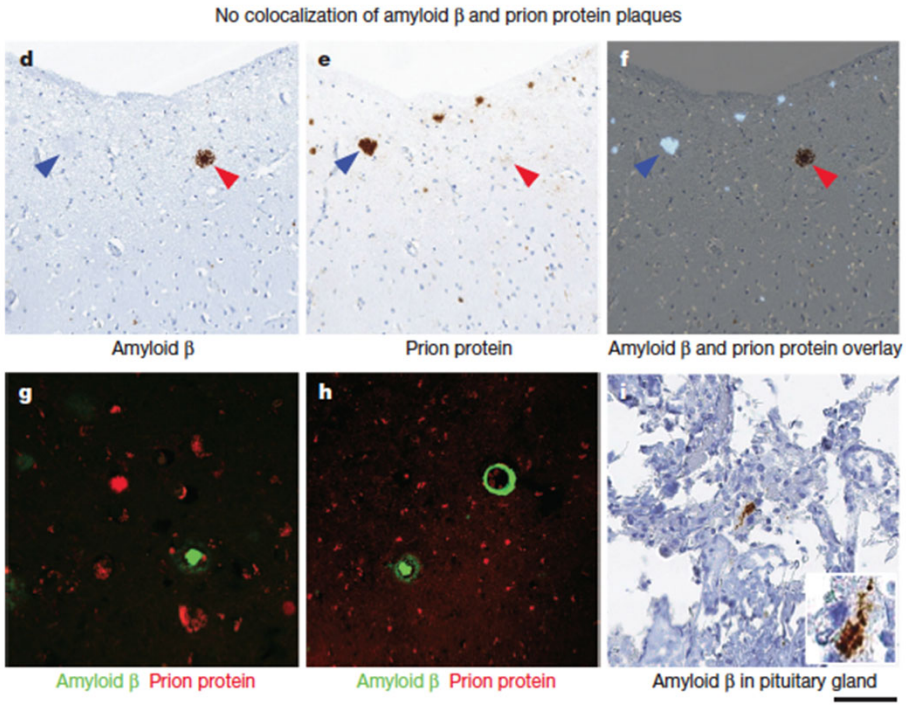
PDK1: therapeutic target for both diseases

Aβ transmitted?

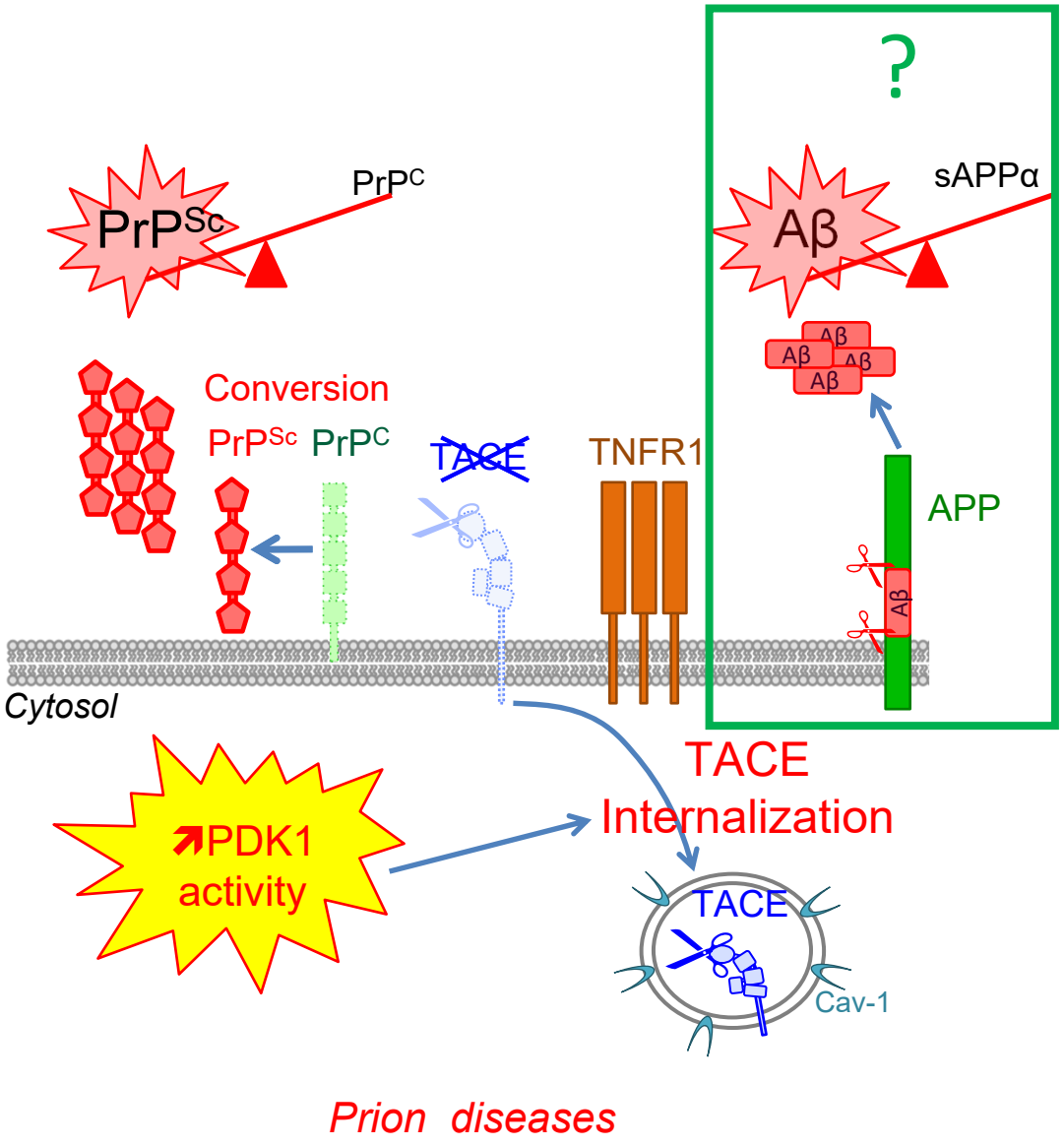


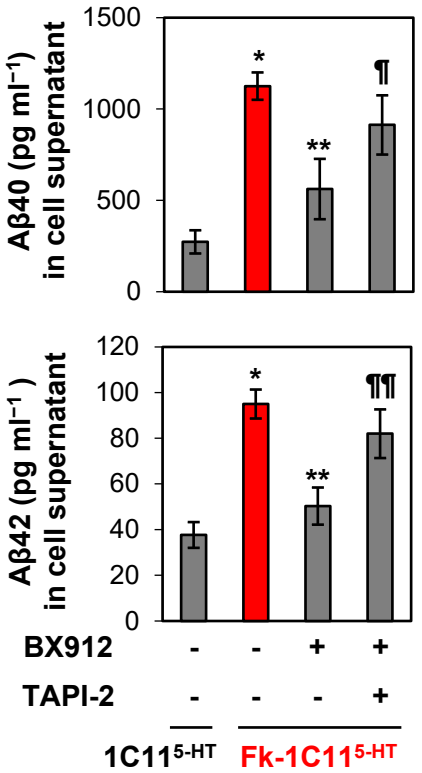
Before 1985, some people treated with cadaver-derived human growth hormone (c-hGH) developped after 30 years a iatrogenic CJD .
These people also had Aβ deposits in the brain, suggestive of incipient Alzheimer's disease.

Hyp: cadaver-derived human growth hormone also contains Aβ seeds => AD transmitted?



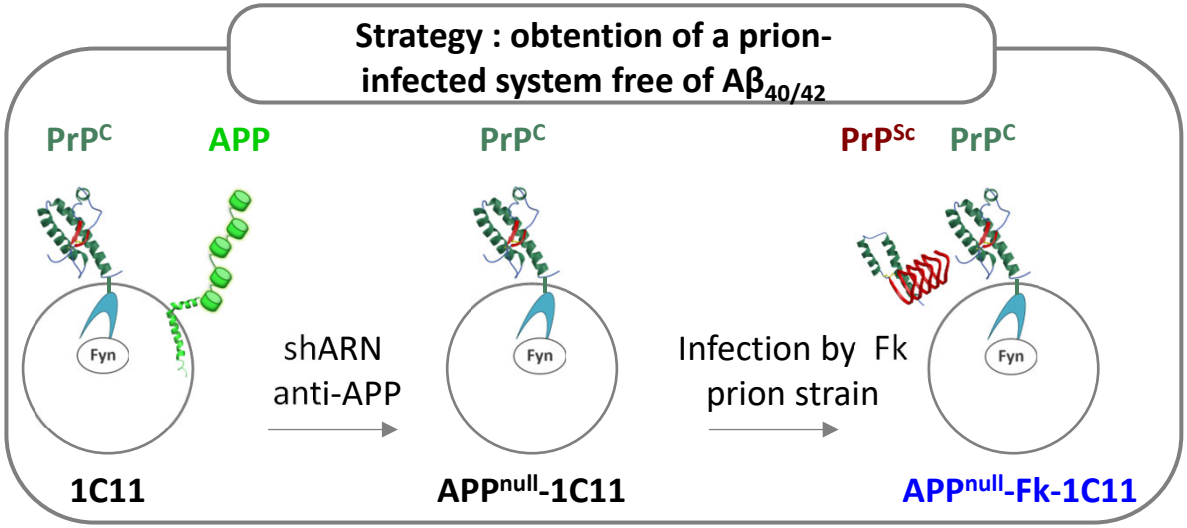
PDK1-dependent TACE uncoupling to APP in prion diseases?

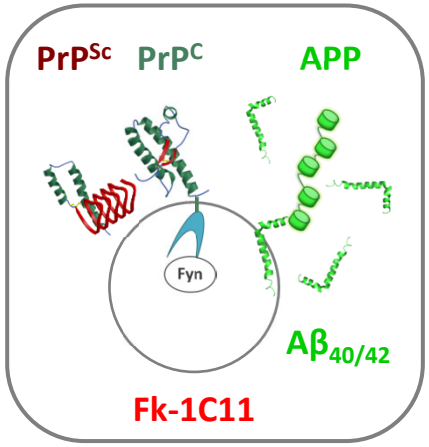




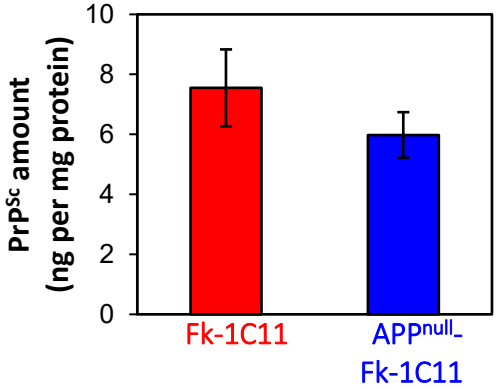
Nature Communication, 2019

What is the impact of Aβ on prion infection *in vitro*?





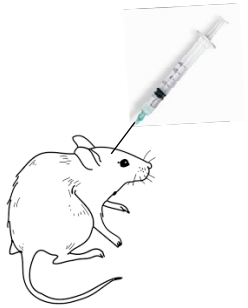
APP depletion does not block prion infection



APP depletion does not affect PrP^{Sc} replication

Nature Communication, 2019

What is the impact of Aβ on prion pathogenesis *in vivo*?



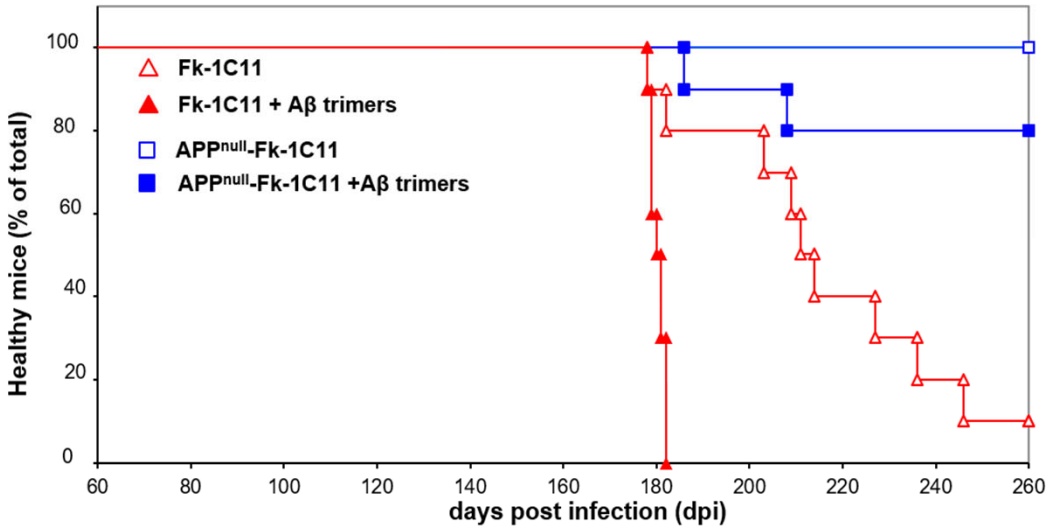
APP^{null}-Fk-1C11
(Aβ free)

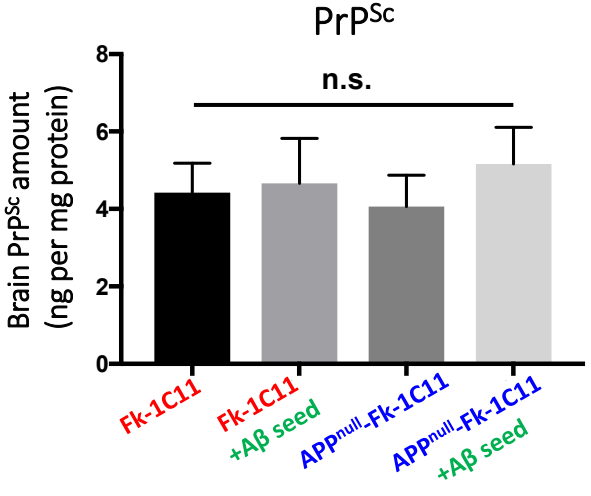
APP^{null}-Fk-1C11
(Aβ free)
+
Aβ seed
(trimers)

Fk-1C11
(containing Aβ)

Fk-1C11
(containing Aβ)
+
Aβ seed
(trimers)

Aβ deposition





⇨ In prion-infected mice:

1. Need of a Aβ seed to induce Aβ deposition
2. Aβ deposition accelerate death

Nature Communication, 2019

TAKE HOME MESSAGE

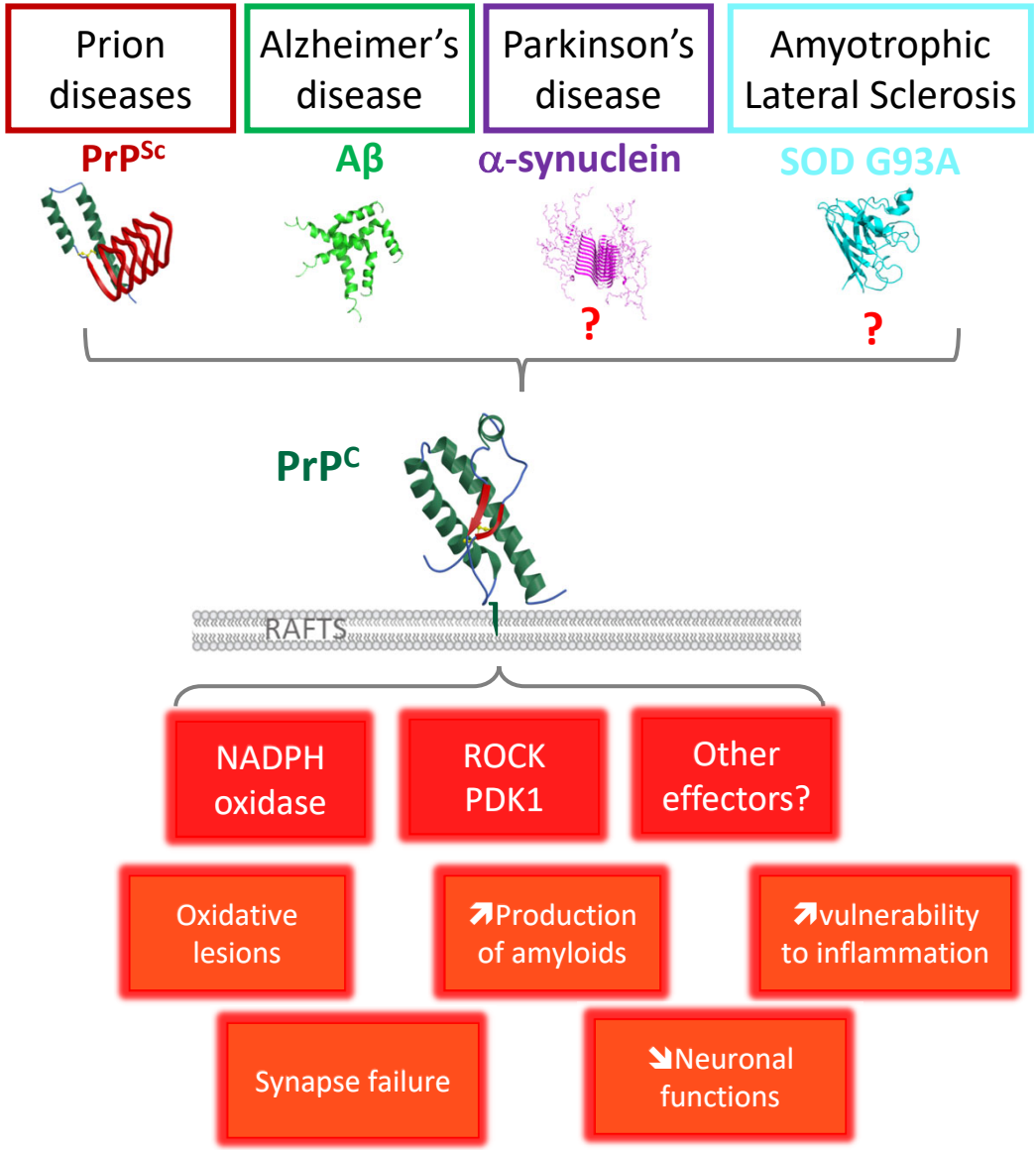
- 1C11 cell line = A stem cell background to tackle basic and clinical challenges relating to neuronal response to antidepressants/ prion - induced neurodegeneration/ neurotoxicity

- Pathological conditions emerge from deregulation of signaling cascade normally dedicated to homeostasis

- Importance of spatio-temporal distribution of signaling effectors

- Prion and Alzheimer's disease share common mechanism of neurodegenerescence : deregulation of PrP^C signaling function

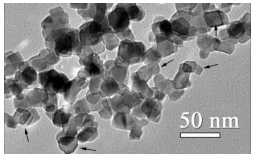
From amyloids to nanoparticles neurotoxicology



Amyloid-based Neurodegenerative Diseases:

- PrP^C = common receptor for other amyloids: α-synuclein (PD), SOD G93A (ALS)?
- Common neurodegenerative signaling pathways?

- PrP^C= Broad spectrum receptor for aggregates ? Nanoparticles?



Size NPs aggregates ~ amyloids

- PrP^C= relay of NPs neurotoxicity?

Role of environmental **nanoparticles** in the etiology/aggravation of neurodegenerative diseases (Alzheimer's disease)?

Particle and Fibre Toxicology , 2022

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Environmental Toxicity
Therapeutic Targets
Cellular Signaling
& Biomarkers

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