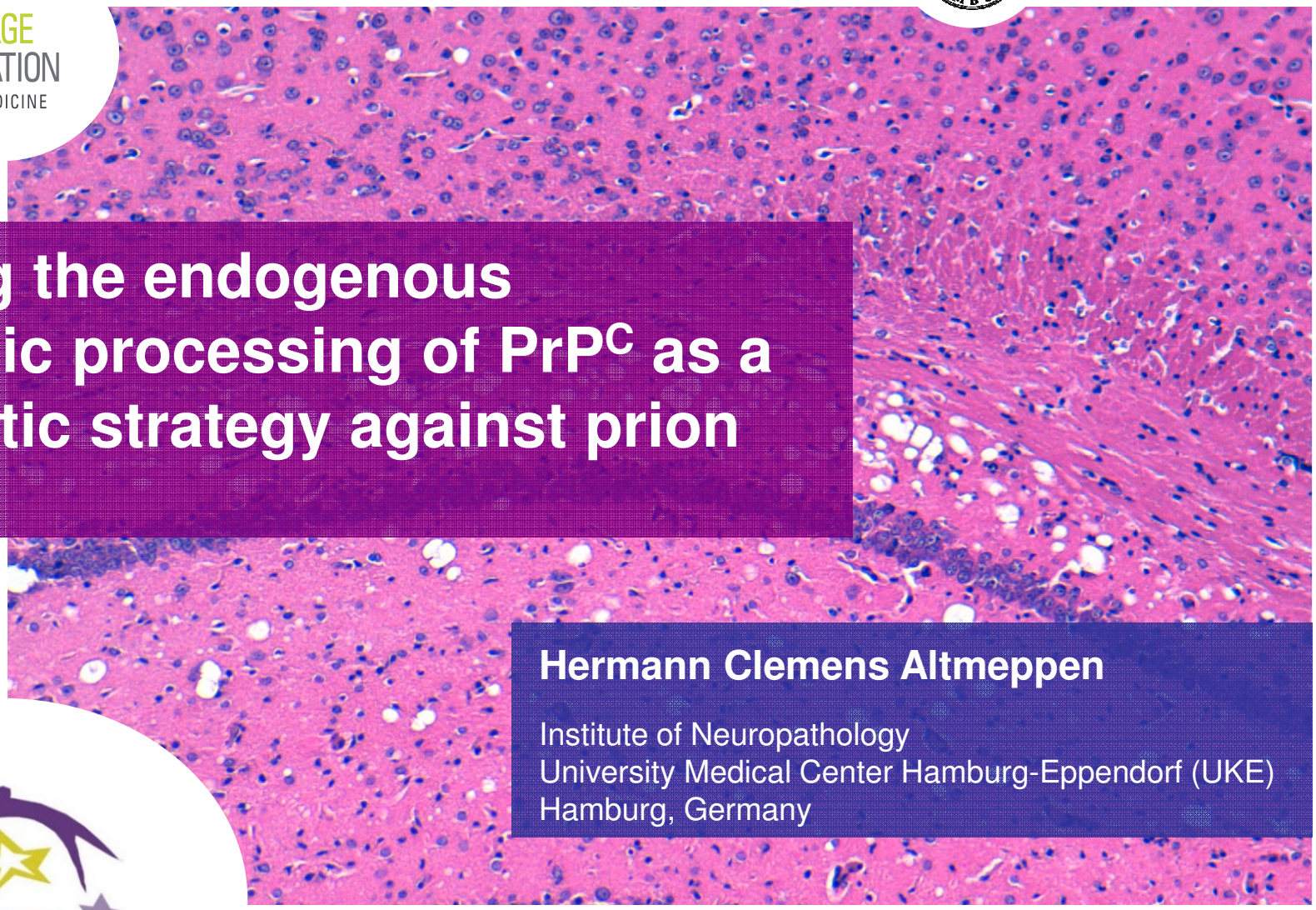
CJD Foundation Family Conference

PROTEIN CLEAVAGE FOR REGULATION
KIEL BIOMEDICINE

Universitätsklinikum Hamburg-Eppendorf



Targeting the endogenous proteolytic processing of PrP^C as a therapeutic strategy against prion diseases

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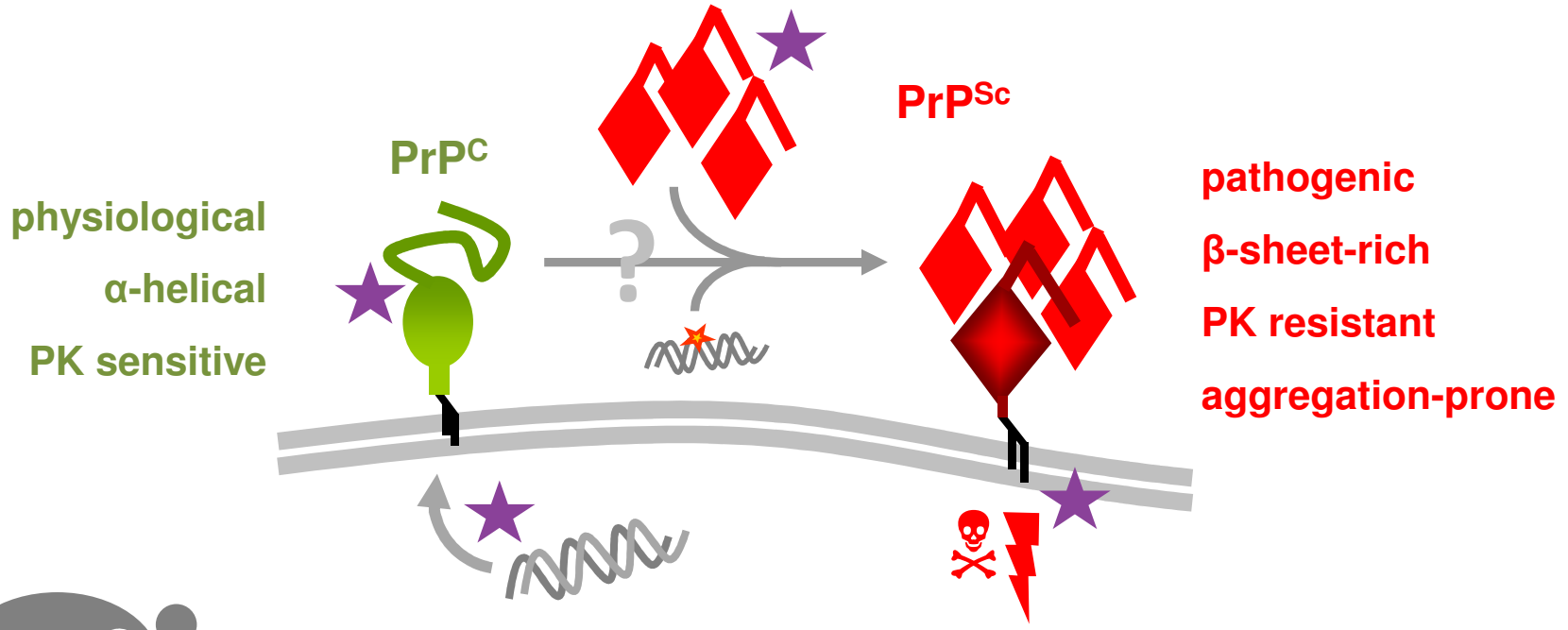
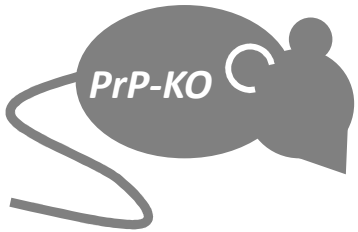
The basis of prion diseases and therapeutic strategies

Self-perpetuating templated conformational conversion („seeded nucleation“)





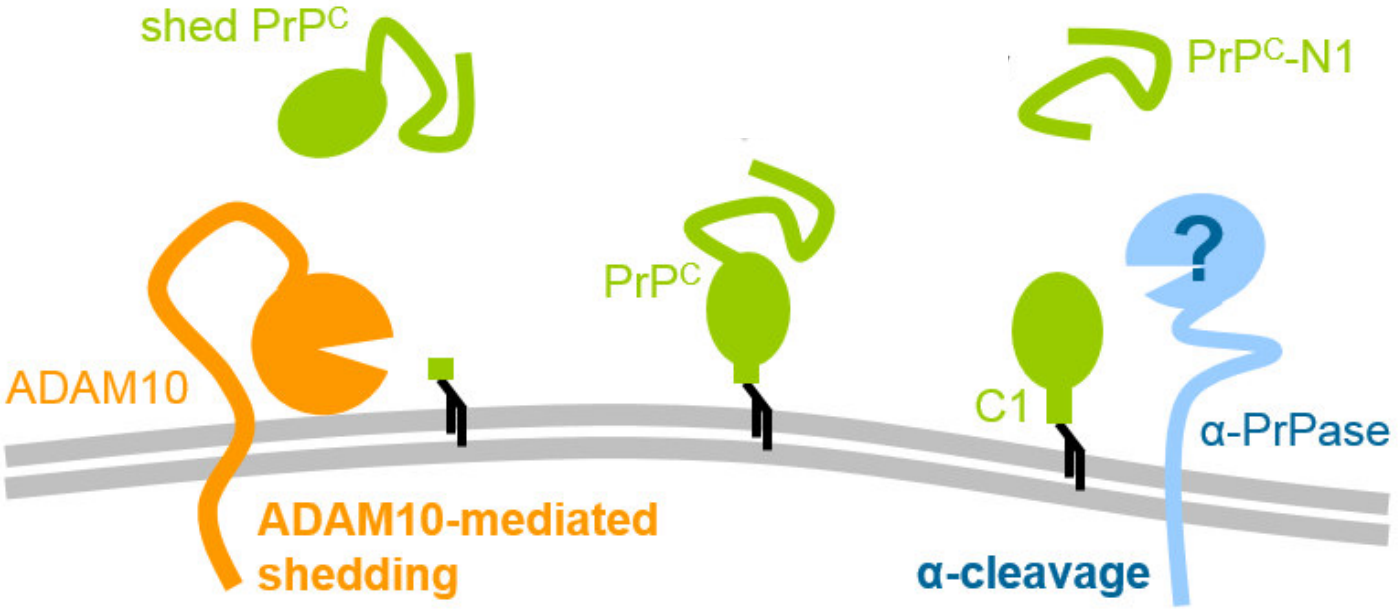
various PrP-knockout mouse models revealed that **PrP^Sc conversion**, **prion spread** and **neurotoxicity** absolutely **depend on PrP^C expression**

★ **therapeutic intervention has been examined at nearly all steps**

Prusiner (1982); Büeler et al. (1992); Brandner et al. (1996); Mallucci et al. (2003); Sandberg et al. (2010); […]

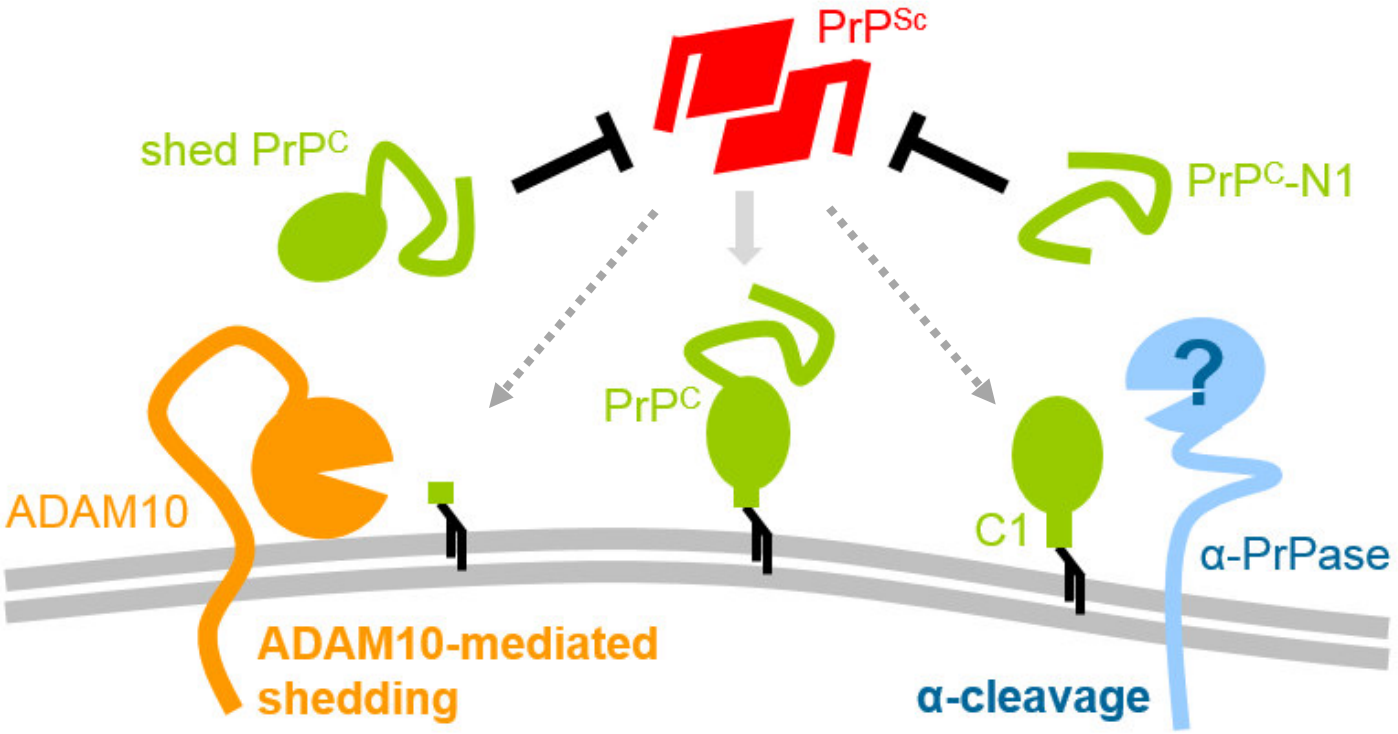
Enzymatic cleavage of PrP^C: Why not taking advantage of a process that is provided by nature?

- proteases are enzymes that cleave proteins
- PrP^C is subject to evolutionary conserved proteolytic cleavages
- physiological and pathological roles of the resulting fragments (?)



Enzymatic cleavage of PrP^C: Why not taking advantage of a process that is provided by nature?

- **reduction of PrP^C at the plasma membrane** { **substrate for PrP^{Sc} conversion** / **receptor / mediator of neurotoxicity**
- **production of protective fragments (?)**

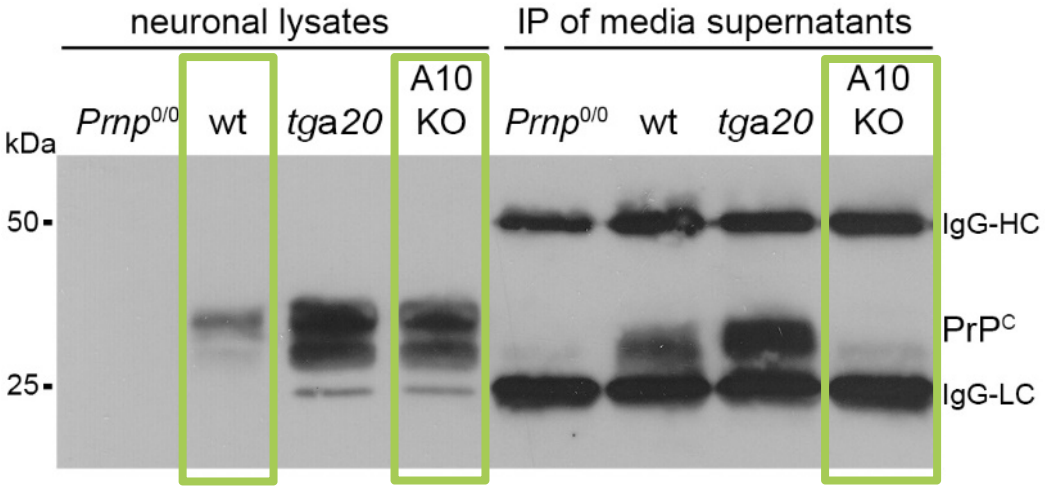


Pharmacological stimulation of the relevant proteases as a therapeutic option

ADAM10 is the functionally relevant sheddase of PrP^C

We have studied the role of ADAM10 in PrP^C biology in different mouse models

Primary neurons of E14 mice:



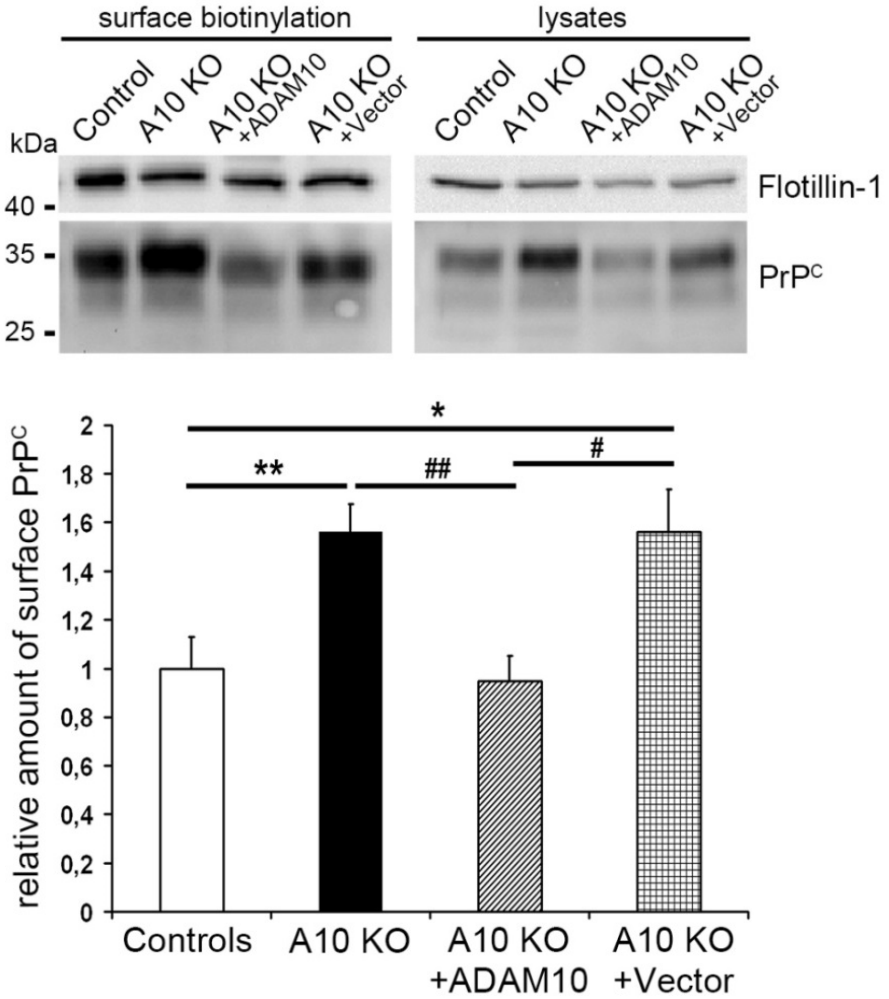
Lack of ADAM10

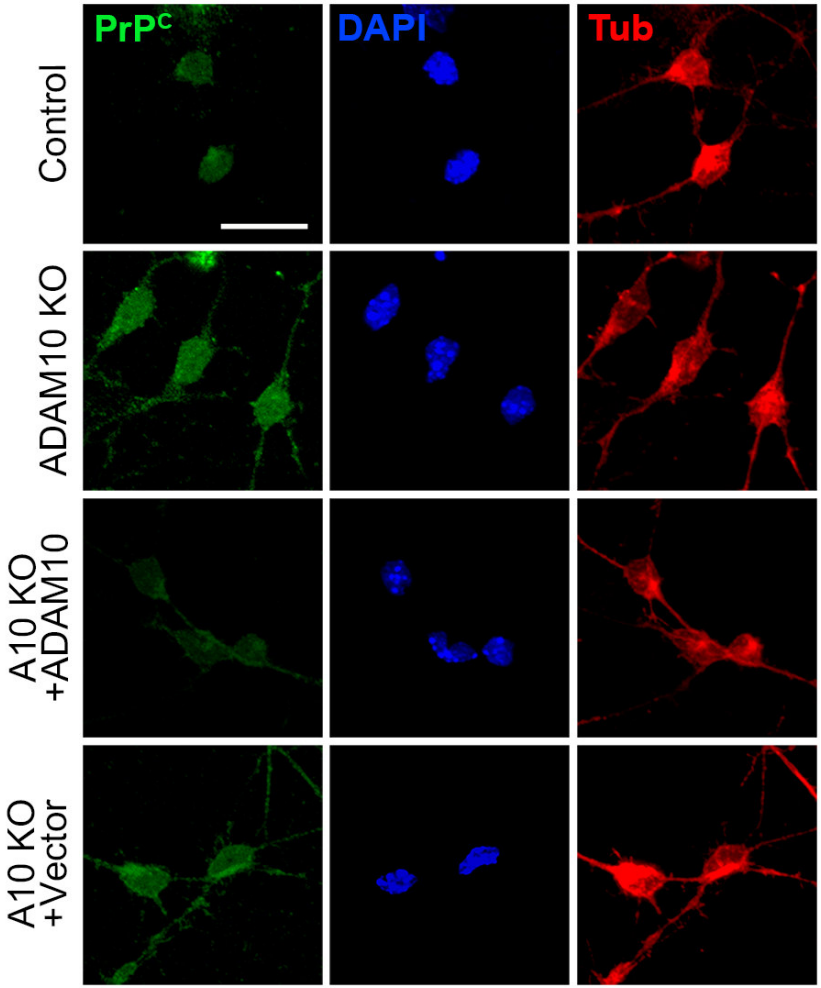
- **abolishes shedding of PrP^C**
- **results in increased PrP^C levels**

Lack of shed PrP might contribute to phenotypes of ADAM10 knockout mice (supporting a protective role)

Hartmann et al. (2002) *Hum Mol Genet*; Jorissen et al. (2010) *J Neurosci*; Altmeppen et al. (2011) *Mol Neurodegener*; Prox et al. (2013) *J Neurosci*

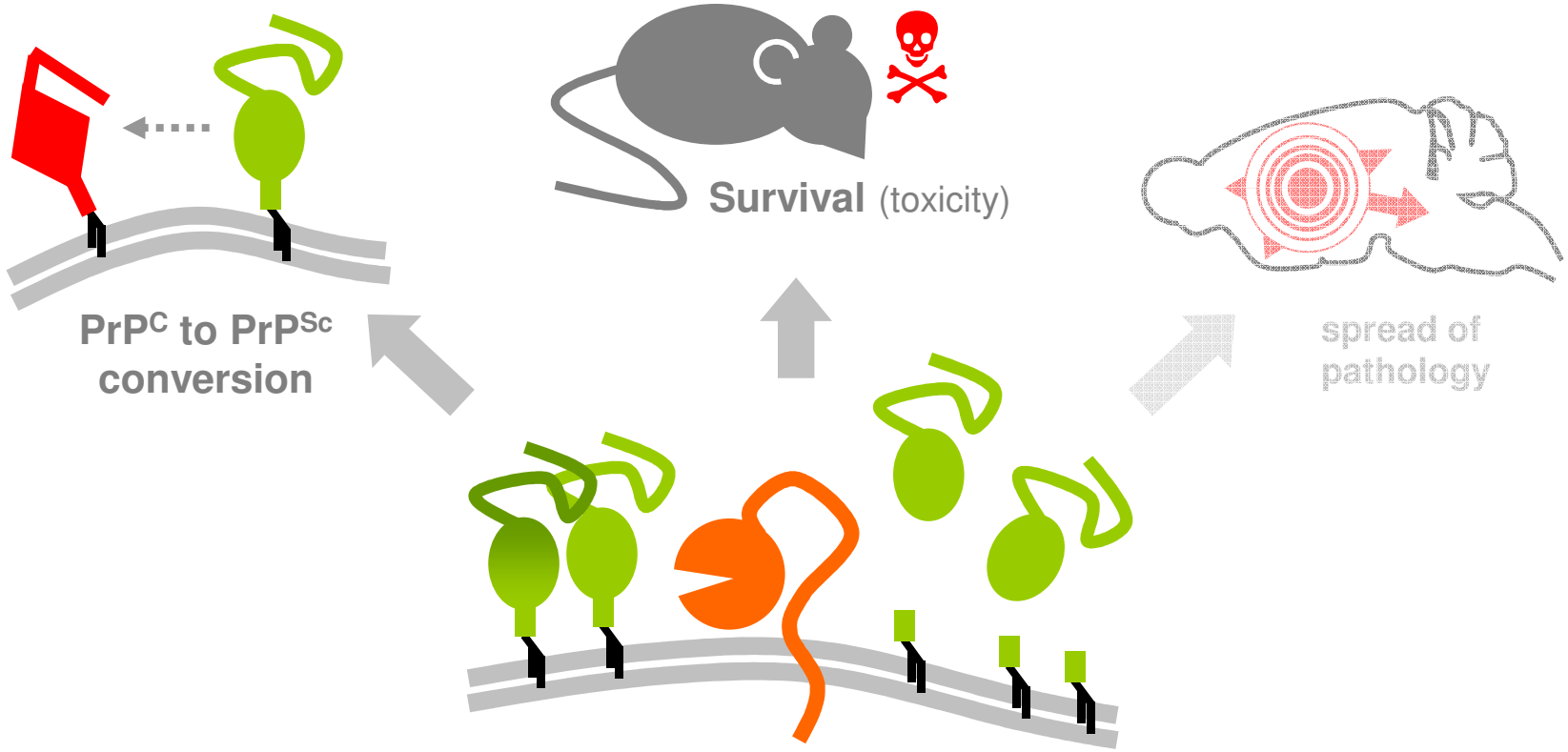
ADAM10 is the functionally relevant sheddase of PrP^{C} and a regulator of its membrane homeostasis





Jorissen et al. (2010) *J Neurosci*; Altmeppen et al. (2011) *Mol Neurodegener*; Prox et al. (2013) *J Neurosci*; Altmeppen et al. (2015) *eLife*

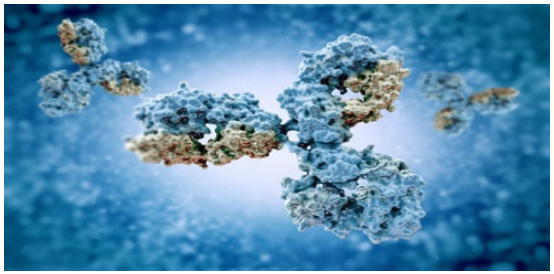
Our most recent ADAM10 KO model revealed that shedding affects several pathomechanistic aspects



In line with a previous study using ADAM10 overexpressing mice (Endres et al., 2009) **these data support a protective role of ADAM10 in prion diseases**

Altmeppen et al. (2015) *eLife*

Glatzel et al. (2015) *Prion*



Direct investigations on PrP^C shedding have been difficult in the past…

Indirect analysis of PrP shedding

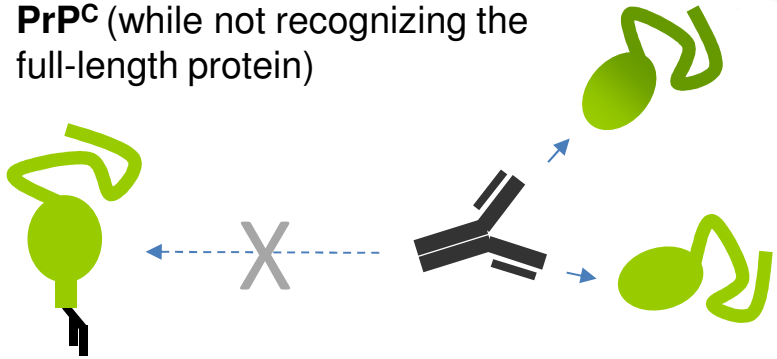
(altered steady-state levels)

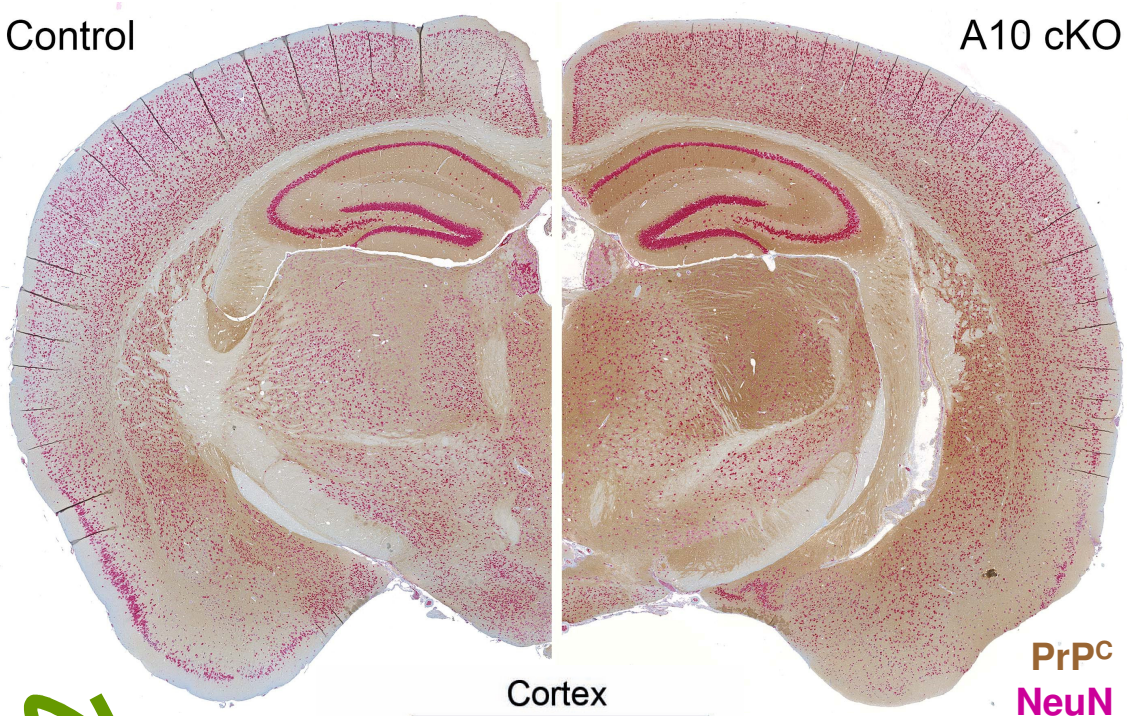
versus

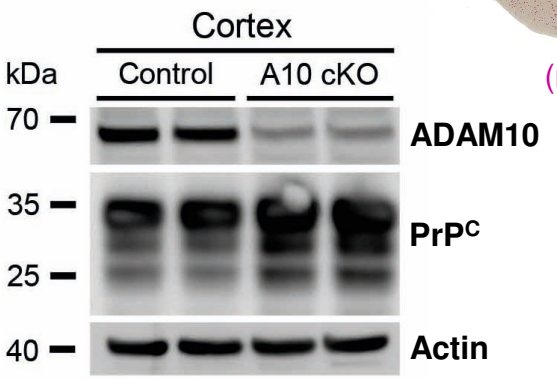
direct assessment of shed PrP

in tissue samples

- **masked by the vast excess of full-length PrP^C**
- **Solution: Generation of an antibody specific for shed PrP^C** (while not recognizing the full-length protein)







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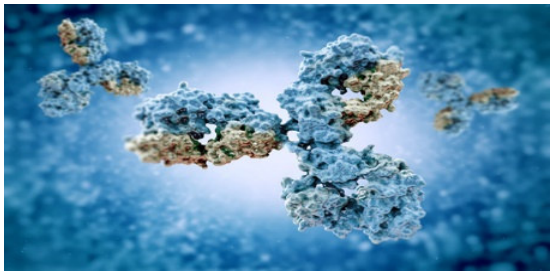
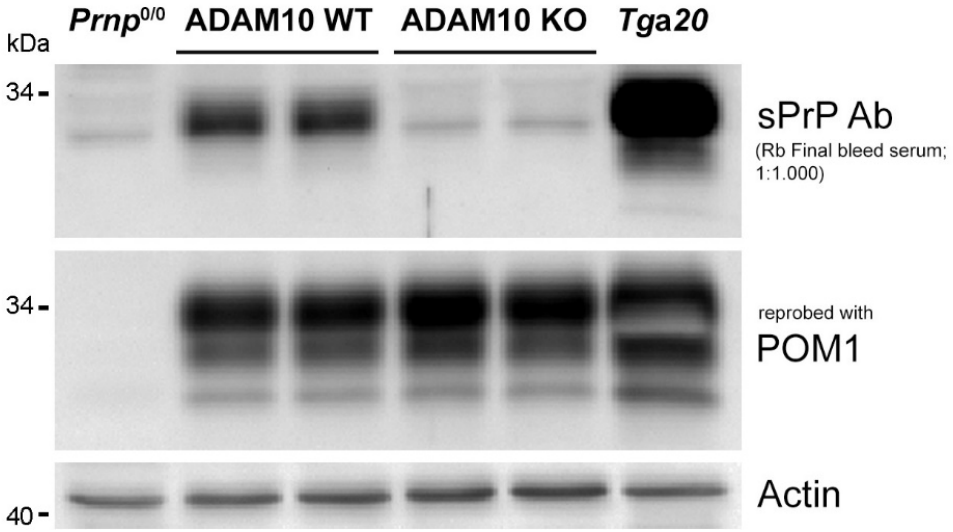


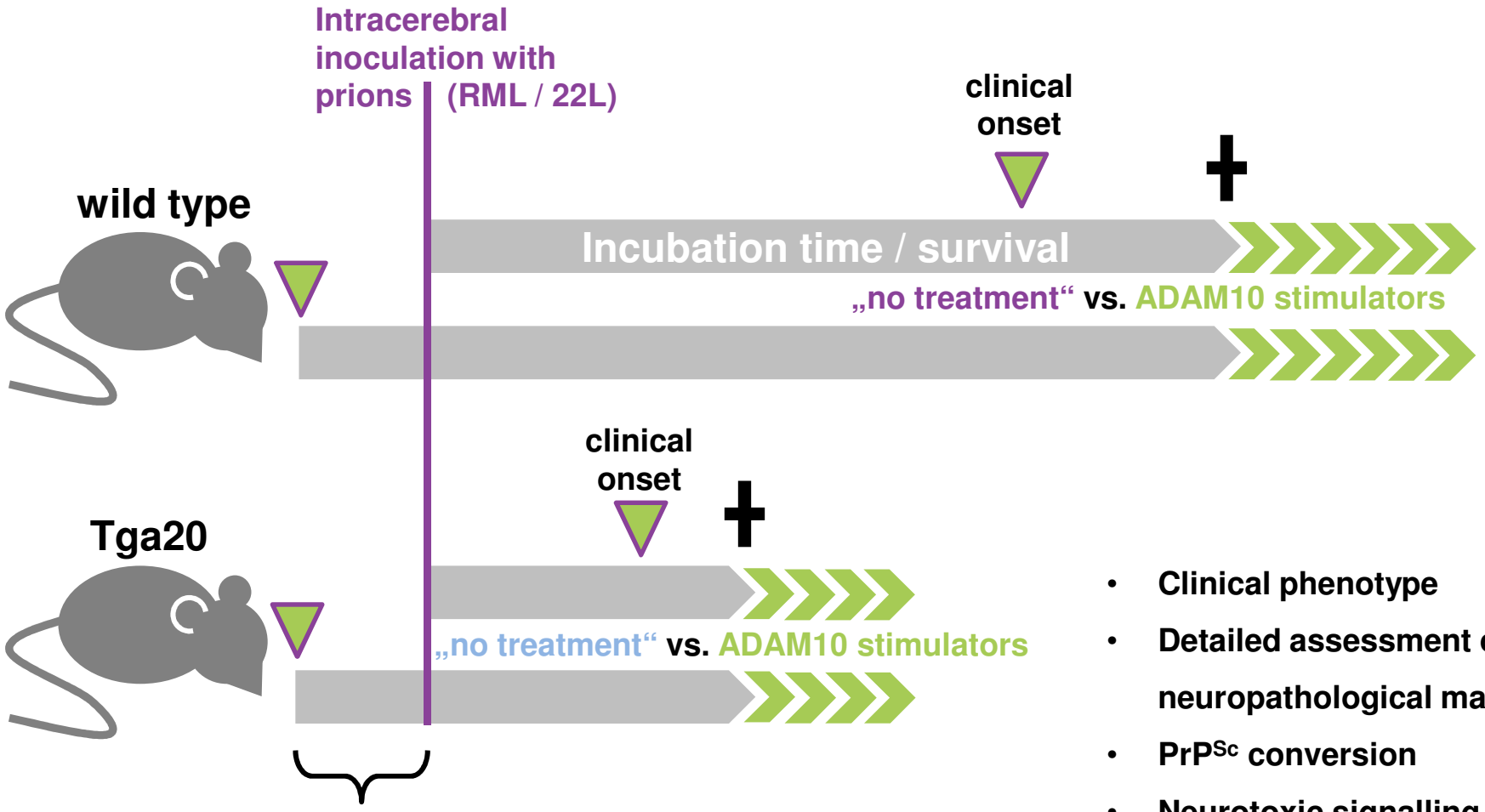
A novel antibody raised specifically against shed PrP will improve our ongoing and future studies



- **all glycoforms can be shed**
  (yet there is a preference for **diglycosylated** PrP^C)
- **lack of ADAM10 is not compensated by another protease**
- **no other cell types seem to contribute to PrP shedding in the brain**
  (self-protection mechanism of neurons?)

With this new antibody we now have a powerful tool to accomplish our proposed study of a pharmacological stimulation of ADAM10-mediated shedding of PrP^C

Stimulation of ADAM10 in prion disease mouse models as a novel therapeutic strategy



- Clinical phenotype
- Detailed assessment of neuropathological markers
- PrP^{Sc} conversion
- Neurotoxic signalling

[…]

A proof-of-concept study: Shed PrP in CSF of patients treated with an ADAM10 stimulator

Pharmacological stimulation of ADAM10 has recently been investigated as a treatment option in Alzheimer`s disease
(Endres et al., 2014, Neurology)

As a **proof of principle study**, we will assess levels of shed PrP (baseline versus treatment) in the liquor (CSF) of the very same patient cohort.

DATA
(under construction due
to ongoing analysis)

Elevated CSF levels of shed PrP upon treatment with the ADAM10 stimulator would clearly support our hypothesis and complement the data obtained in mice

Outlook

- **Cell culture based studies on the mechanistic finetuning of PrP^C shedding**
- **Investigating the physiological functions of shed PrP**
- **Identification of the protease(s) responsible for the α-cleavage of PrP^C**
- **Combination therapy – Stimulation of PrP cleavage PLUS PrP^C stabilizing compouds**
- **Rational drug design (improved performance & specificity of stimulators)**

Summary

- Despite enormous efforts and relevant progress during the past decades there is still no effective treatment against prion diseases
- The prion protein (PrP^C) is cleaved by endogenous proteases (i.e. protein-cleaving enzymes)
- Cleavage of PrP by such enzymes has been shown to significantly impact on prion diseases
- ADAM10 is a protease that releases nearly full-length PrP from the plasma membrane. Lack of ADAM10 in mouse brains leads to drastically shortened survival time upon prion infection. Overexpression of ADAM10, in contrast, results in prolonged survival.
- Activity of enzymes can be manipulated and pharmacological stimulation of ADAM10-mediated shedding of PrP^C is investigated as a novel treatment option against prion diseases