The Role of Cellular Chaperones and the Unfolded Protein Response in GSS Syndrome

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Telling Lab

Prion protein (PrP): a known cellular factor

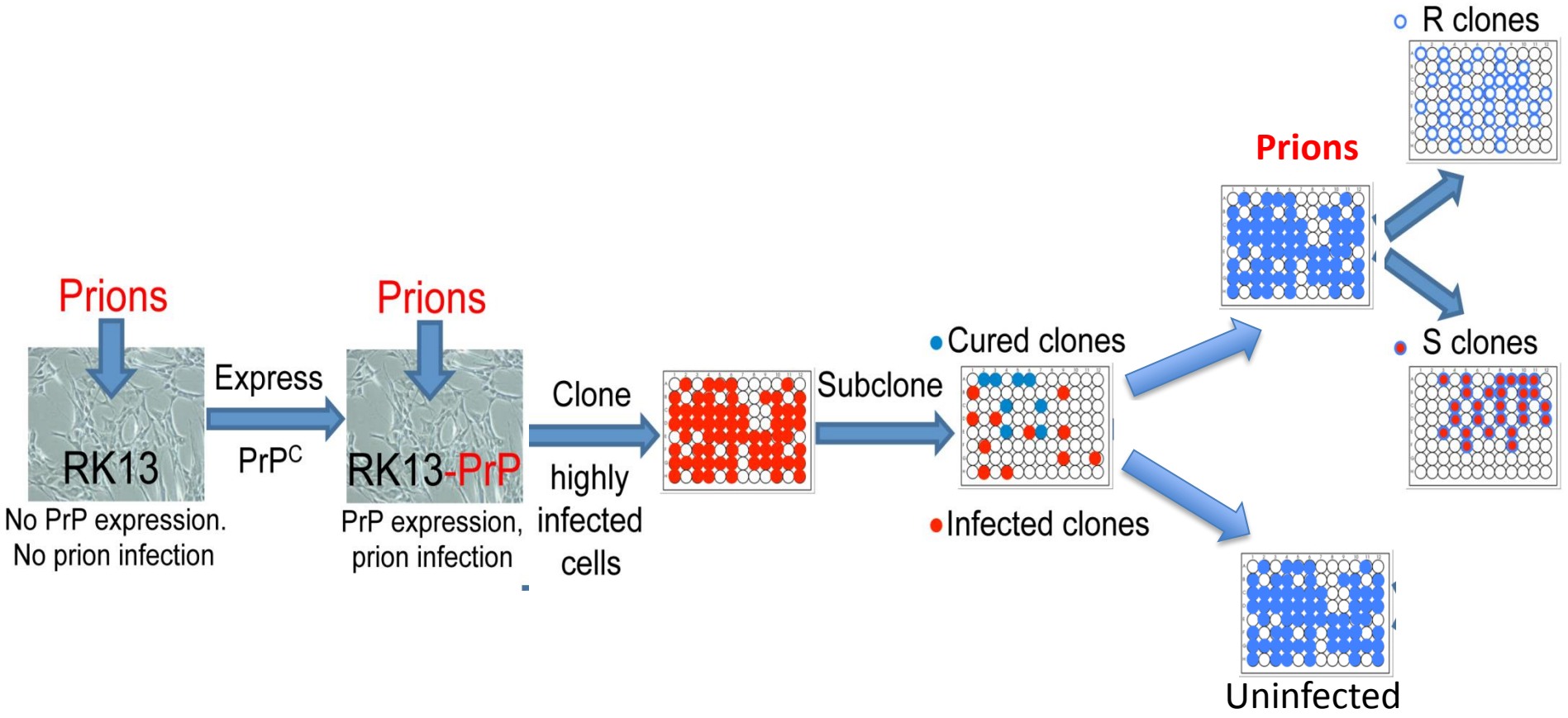
- PrP null mice are unable to replicate and produce disease when exposed to infectious prion materials (Brandner et al., 1996; Bueler et al., 1993; Fischer et al., 1996; Manson et al., 1994; Prusiner et al., 1993)
  - Removal of cellular PrP (PrP^C) can reverse pathogenesis and is neuroprotective (Mallucci et al., 2003; 2007; White et al., 2008)

- However, not all cells are susceptible to prion infection even with PrP expression
  - Some prion susceptible cells have sub populations that are resistant as well

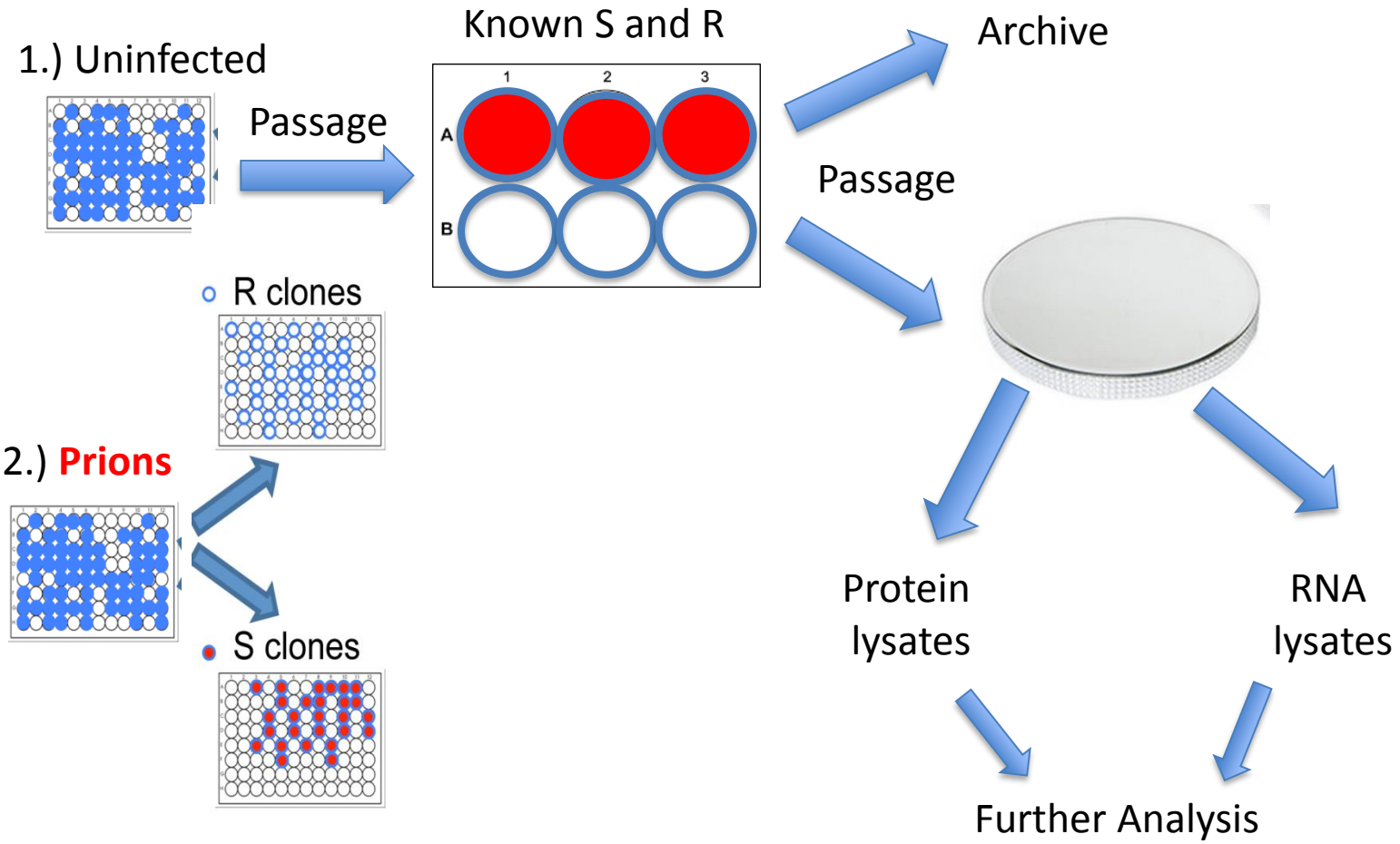
Hypothesis

- In addition to the prion protein, additional unidentified cellular factors regulate disease susceptibility and pathogenesis

Generation and Identification of susceptible (S) and resistant (R) cells



Experimental Set-up: Further Analysis of uninfected S and R cells



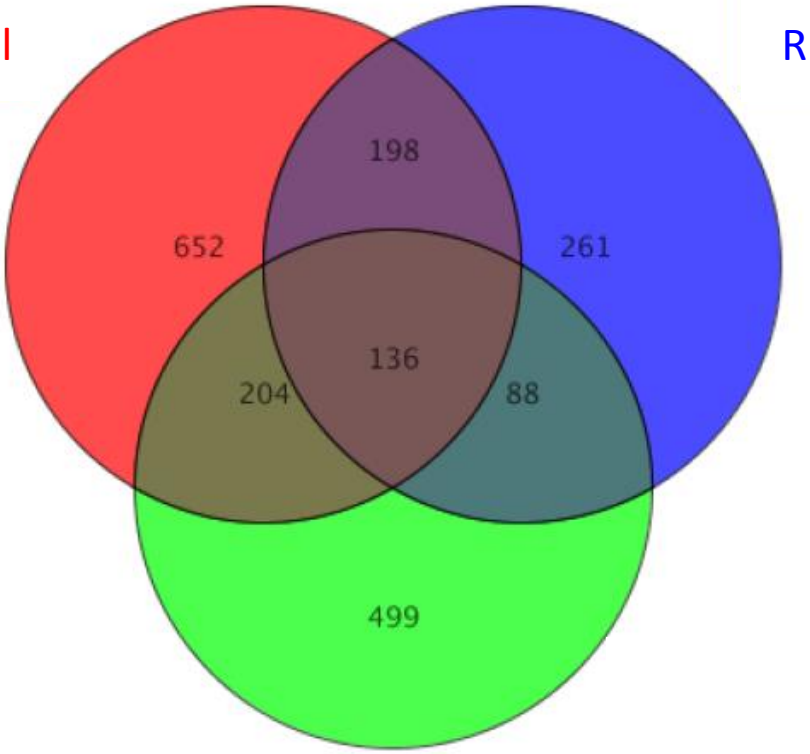
Questions

- What genes/cellular pathways are responsible for prion susceptibility?
- Is there a unique genetic signature dependent on the specific PrP strain? Or generic?

Identification of differences between S and R cells

RK13-ElkPrP differential expressed genes

RK13-Deer PrP differential expressed genes



108 known genes (80%)
28 ‘new’genes (20%)

Combined RK13- Deer, Elk and OvinePrP differential expressed genes

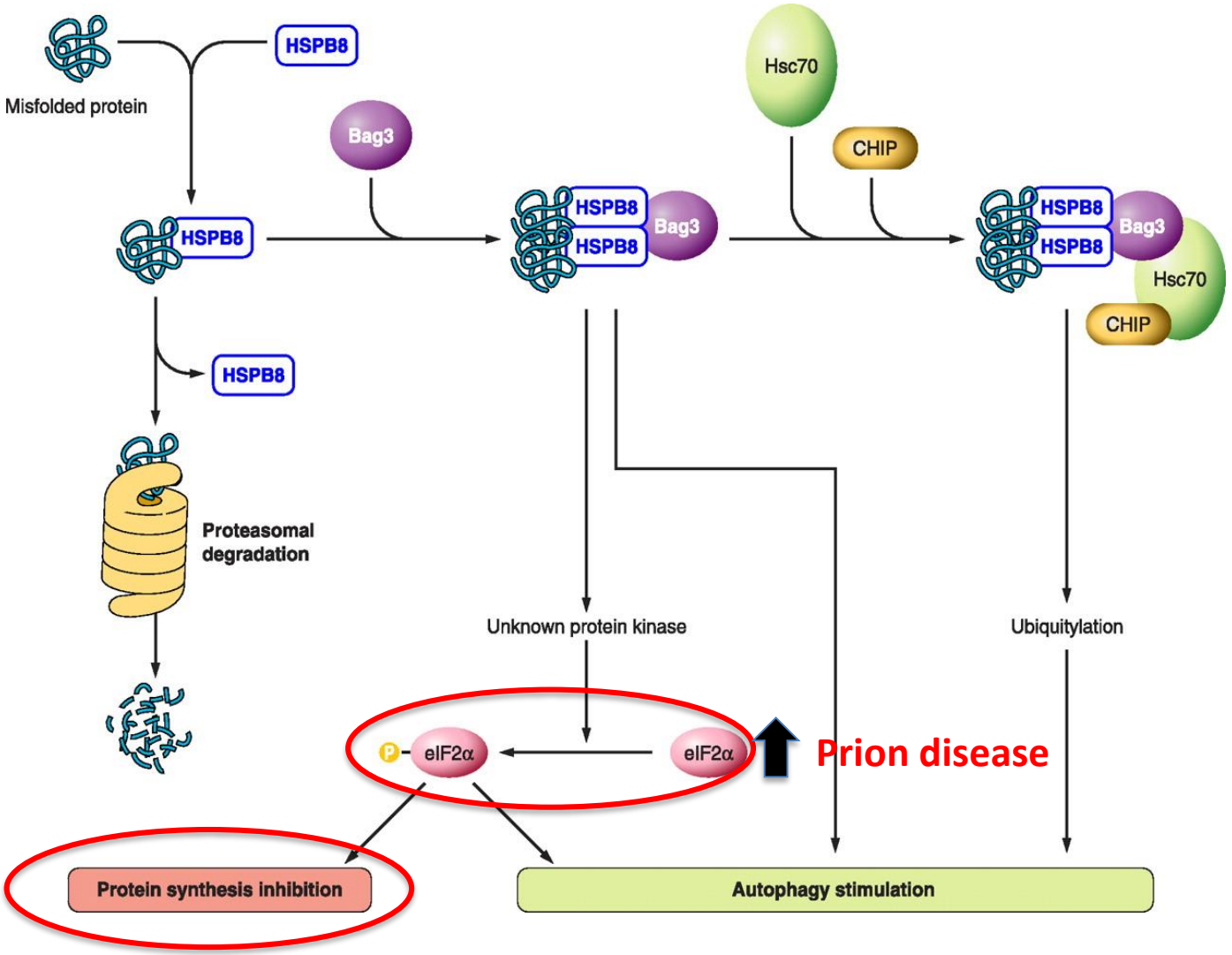
Small heat shock protein or chaperone: HSPB8

- With the co-chaperone BAG3 is known to activate and recruit autophagic machinery in protein folding disorders (Carra et al, 2008 JBC ; Crippa et al., Hum Mol Gen 2010)

- Found upregulated in Alzheimer's (AD), Parkinson's (PD), Huntington's (HD) and spinocerebellar ataxia type 3 (SCA3) diseased patient brains specifically in astrocytes (Seidel et al., 2012 Neuropath and Appl Neurobio.)

- Inhibits protein synthesis though the P-eIF2α stimulating autophagy (Carra et al, 2009 JBC)

Participation of HSPB8 in regulated proteolysis of misfolded proteins



Mymrikov E V et al. Physiol Rev 2011;91:1123-1159

Physiological Reviews

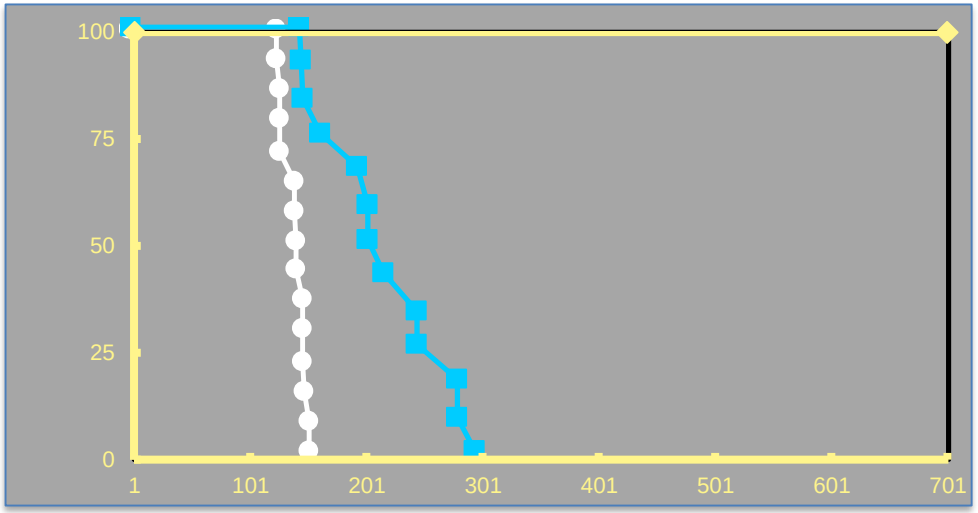
Objectives

- Assess cellular changes of HSPB8 and the UPR during disease
- Determine the role of HSPB8 on prion protein misfolding and pathogenesis
- Investigate the modulation of HSPB8 levels in cells and TgGSS mice

Spontaneous neurodegenerative disease in Tg(GSS)mice

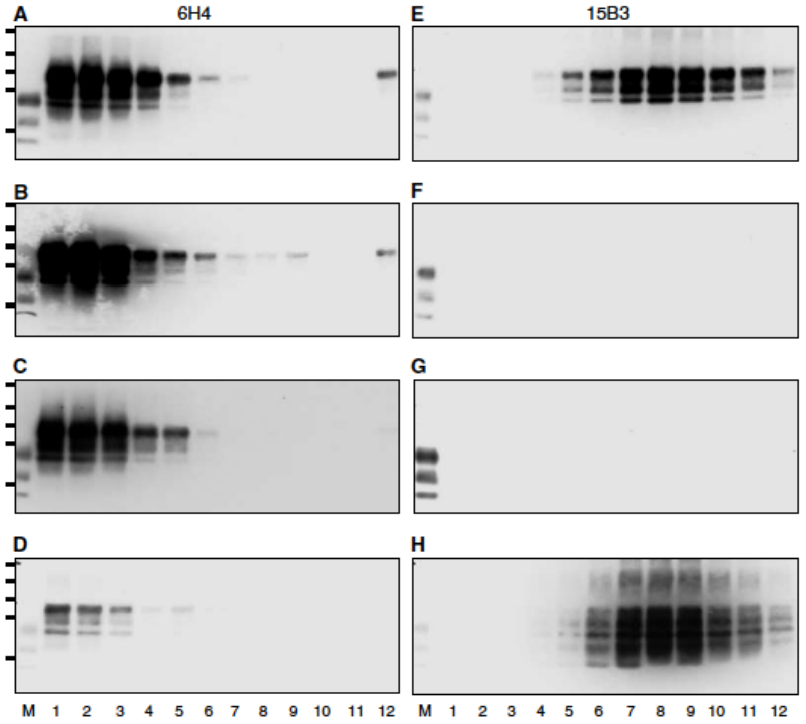
Tg(MoPrP-P101L)-Tg(GSS)22

Tg(MoPrP)



Tg(MoPrP-P101L)

220±10 (18/18)



Spongiform degeneration

Astrocytic Gliosis

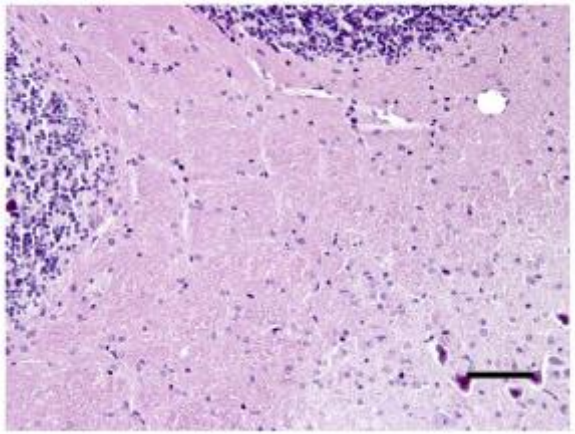
Amyloid deposition

Nazor et al., 2005

Spontaneous neurodegenerative disease in Tg(GSS)mice

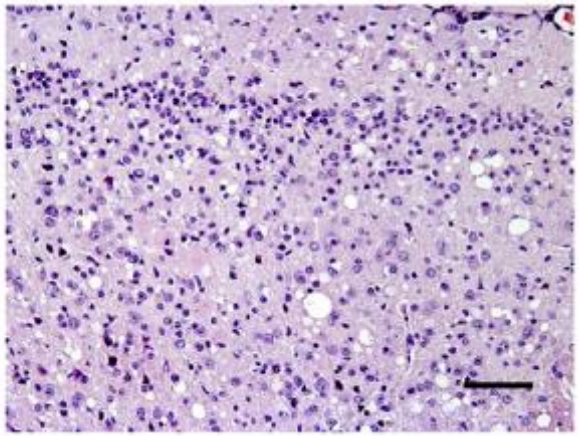
Cerebellum

A



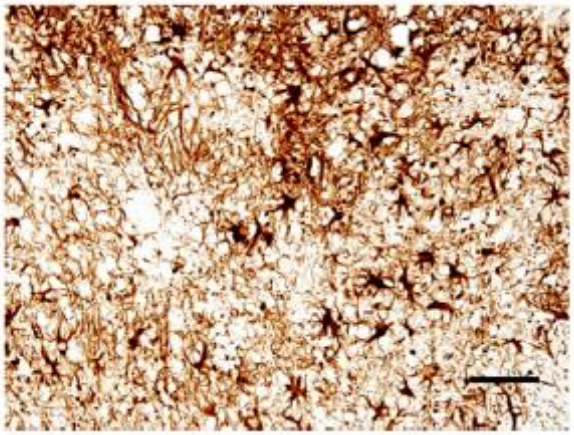
Cortex

B

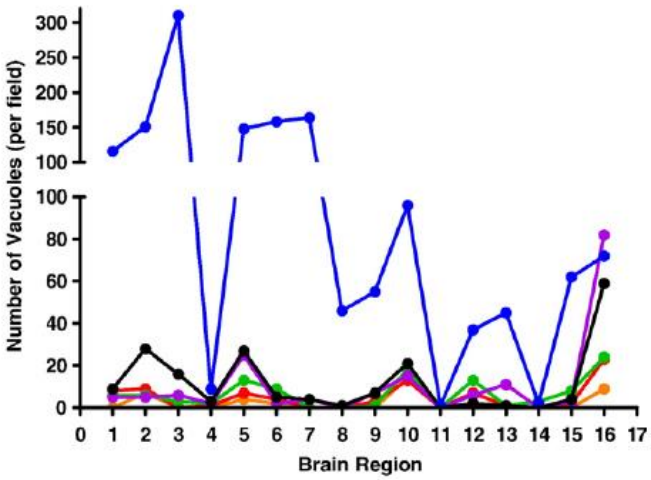


Cortex-GFAP

C

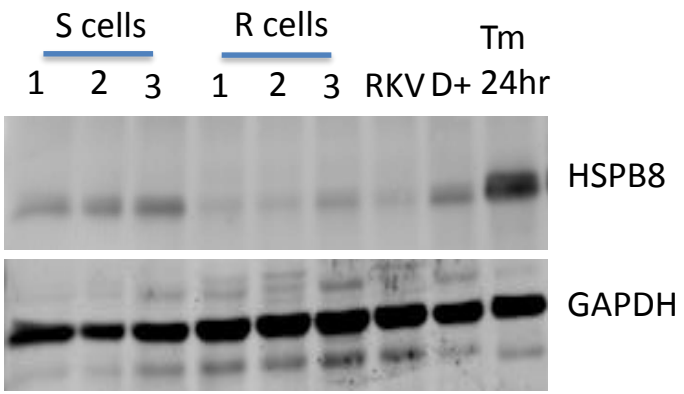


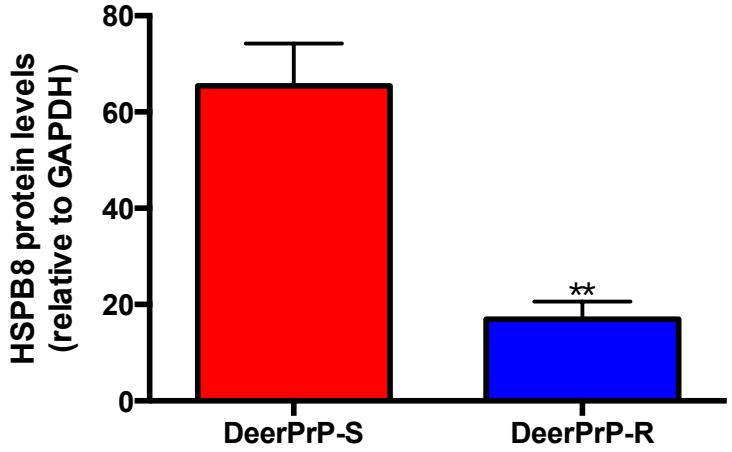
D

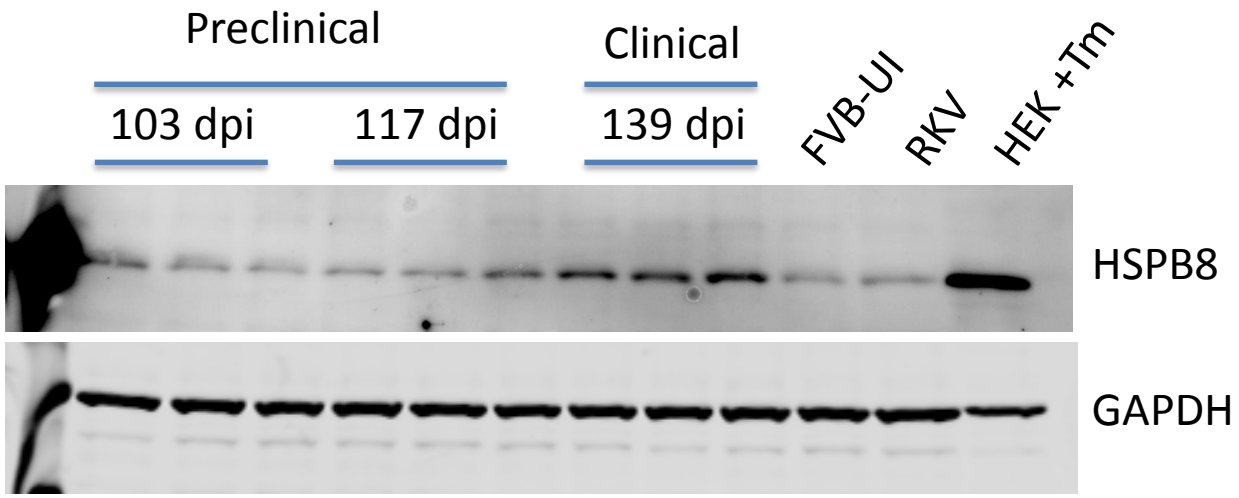


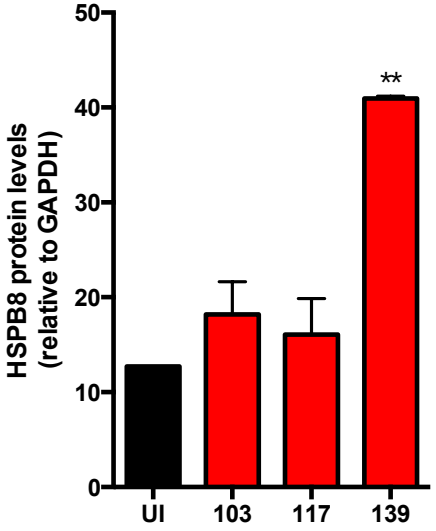
Nazor et al., 2007

HSPB8 protein levels rise in susceptible cells and with clinical disease

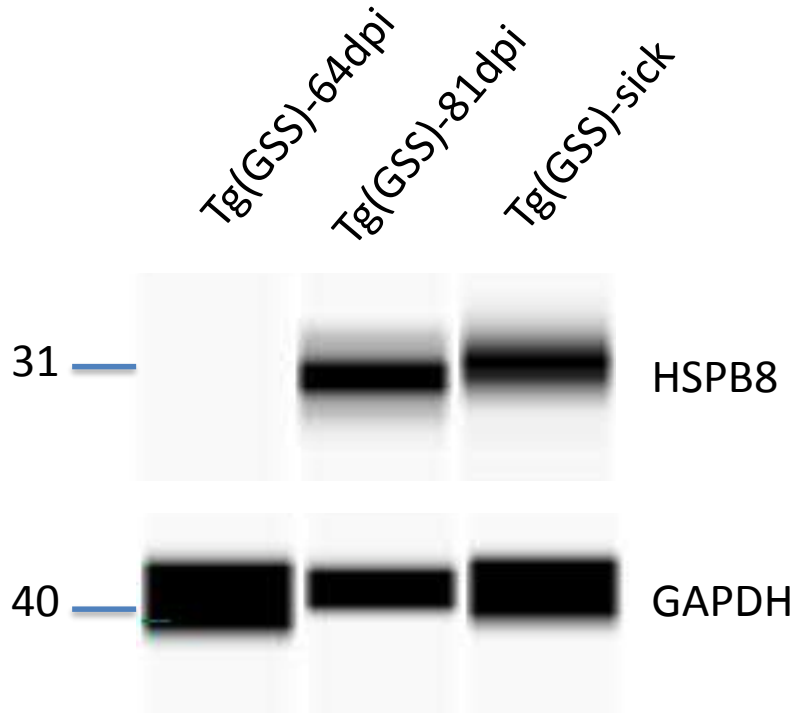








HSPB8 levels increase overtime in Tg(MoPrP-P101L) GSS mice

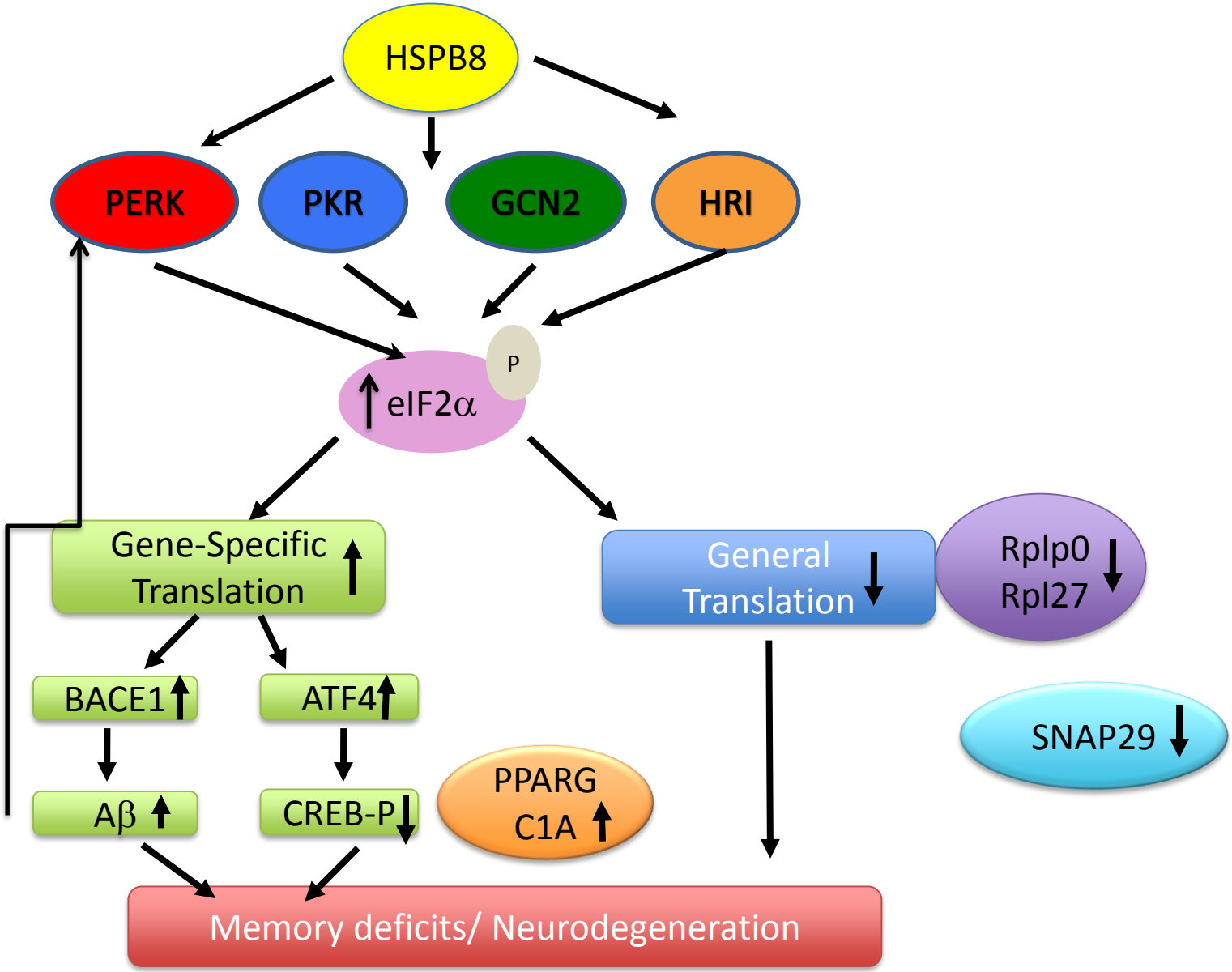


Role of HSPB8 in susceptibility and disease

- HSPB8 is increased in:
  - cells susceptible to prions
  - Mice infected with prions
  - Tg(GSS) genetic mouse model of prion disease

- What is the mechanism behind HSPB8 changes?

HSPB8 and Unfolded Protein Response (UPR)

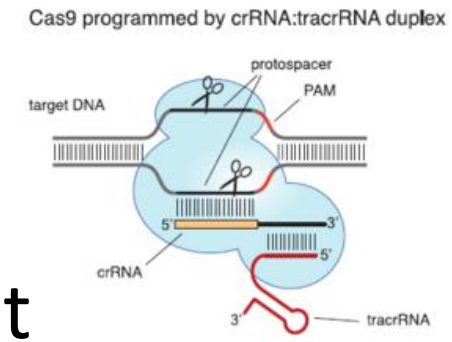


Conclusions

- Established resistant cell lines that express PrP however lack ability to be infected
  - Identified candidate genes and pathways
- HSPB8 levels rise in susceptible cells, prion infection and during Tg(GSS) disease progression
- eIF2α-P or activation occurs during disease progression in Tg(GSS) mice

Future directions

- Continue analysis of HSBP8 and the UPR in Tg(GSS) mice
- Modulate genes/pathways using genome editing and expression constructs
- Continue to identify genes important to prion susceptibility



Acknowledgements

Glenn Telling
Richard Bessen

Telling Lab:
Current members
Jifeng Bian
Hae Eun Kong
Sehun Kim
Vannessa Selwyn
Delaney Swindle
Matt Sabel
Michael Young
James Dilisio
Colin Hastings
Tim Koh
Colin Wakeman
Maddie Harmon

Former members
Jeff Christiansen
Crystal Reid
Vadim Khaychuk
Carla Calvi

Funding
CJD Foundation Grant
R21 NS091027