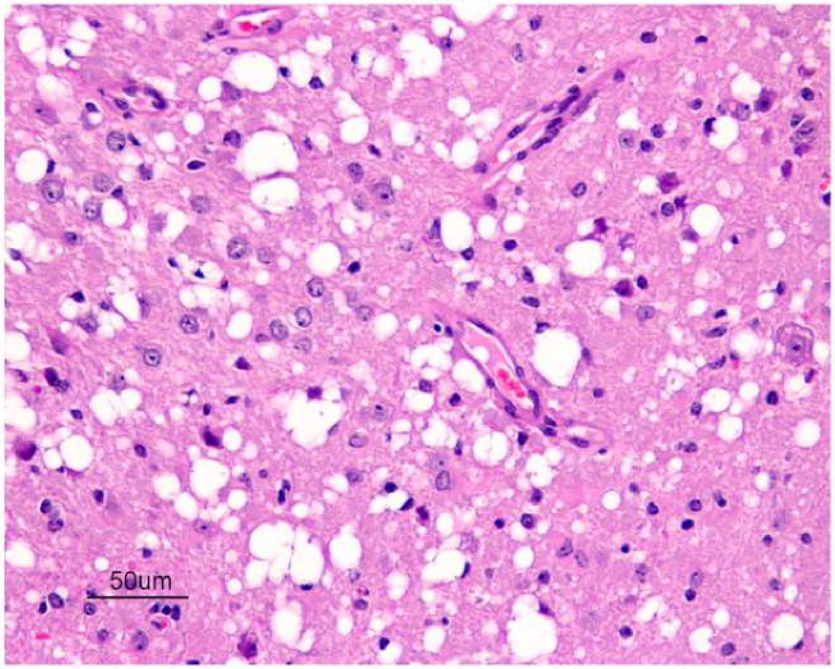
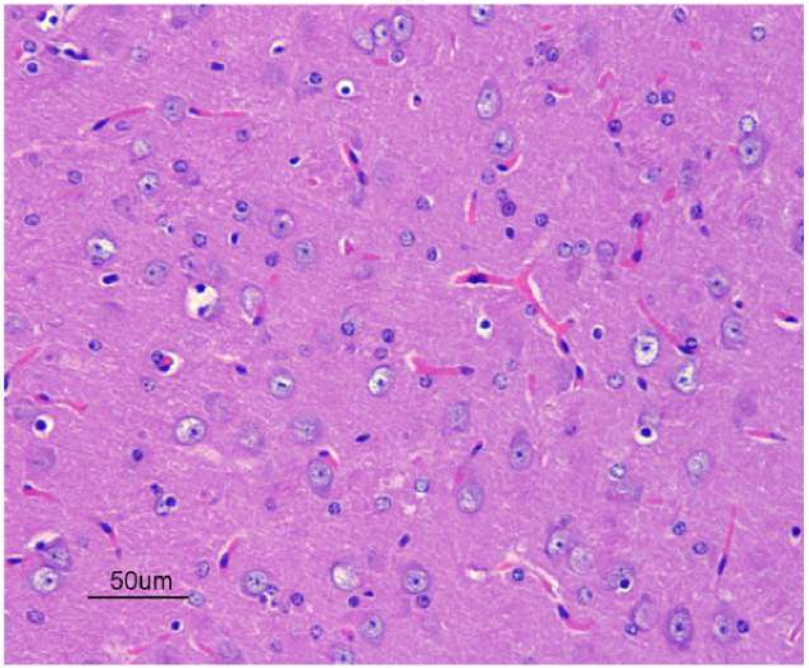
Prions

Transmissible Spongeform Encephalopathies (TSE's)

Diseased brain histology

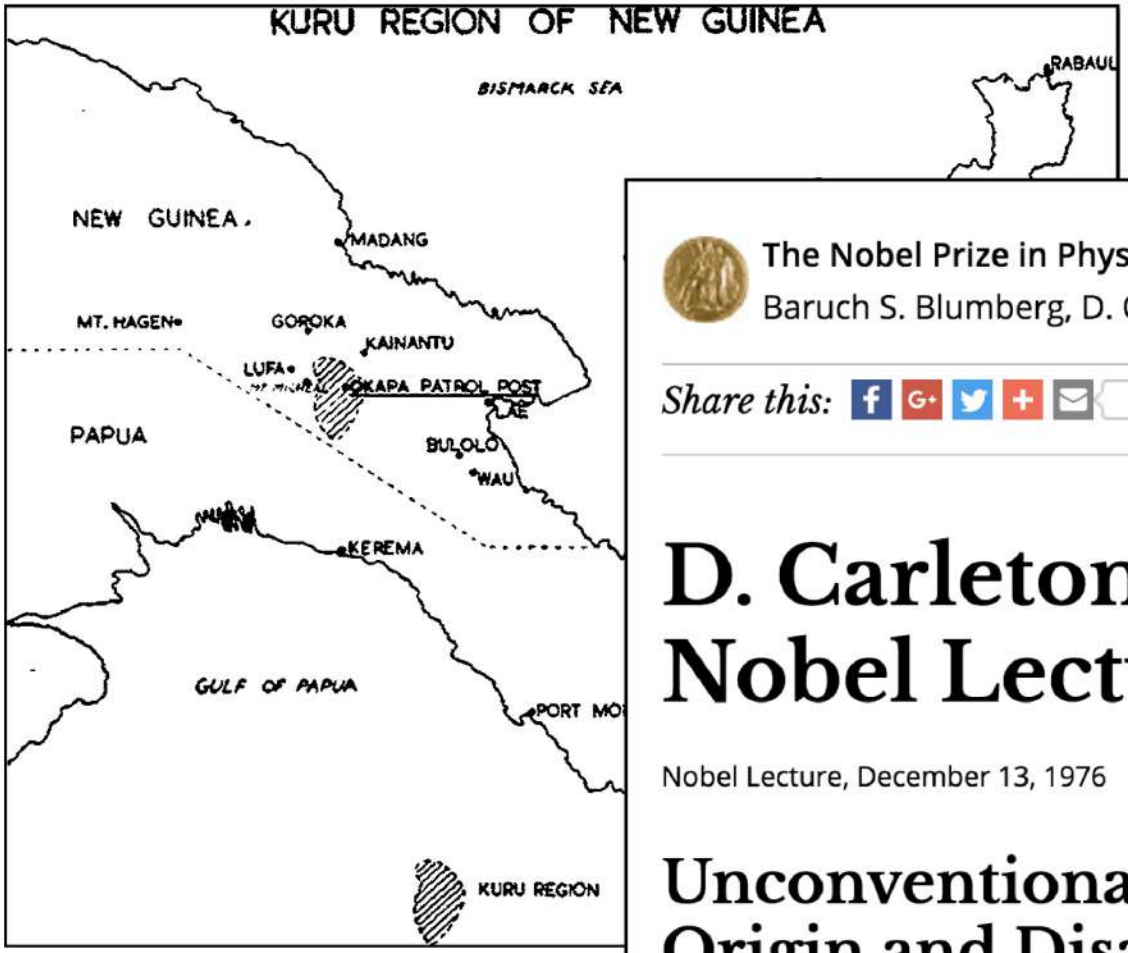


Normal brain histology



TSE	Affects
Scrapie	Sheep/Goats
Bovine Spongeform Encephalopathy	Cow
Chronic Wasting Disease	Deer/Elk
Creutzfeldt-Jakob Disease	Humans
Fatal Familial Insomnia	Humans
Kuru	Humans (ritualistic cannibalism)

Kuru



The Nobel Prize in Physiology or Medicine 1976

Baruch S. Blumberg, D. Carleton Gajdusek

Share this: 0

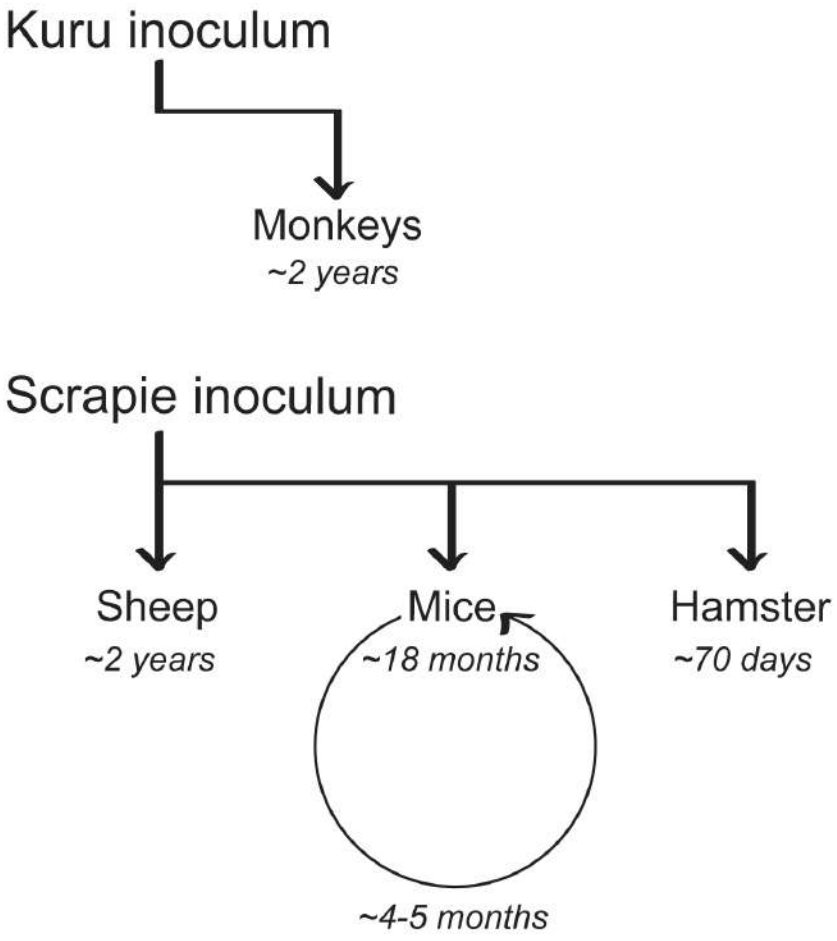
D. Carleton Gajdusek - Nobel Lecture

Nobel Lecture, December 13, 1976

Unconventional Viruses and the Origin and Disappearance of Kuru

(Gajdusek and Zigas, 1957)

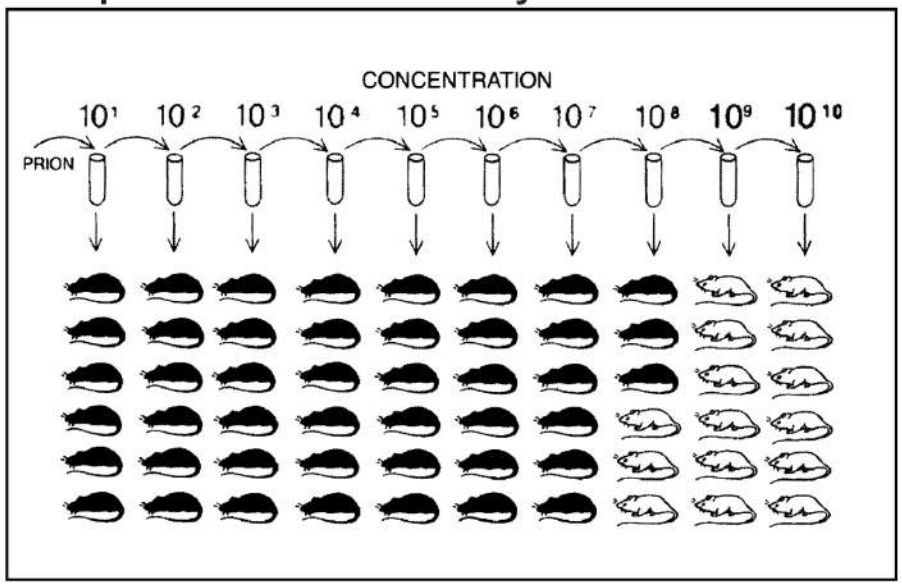
Developing a model system to ID the ‘scrapie agent’



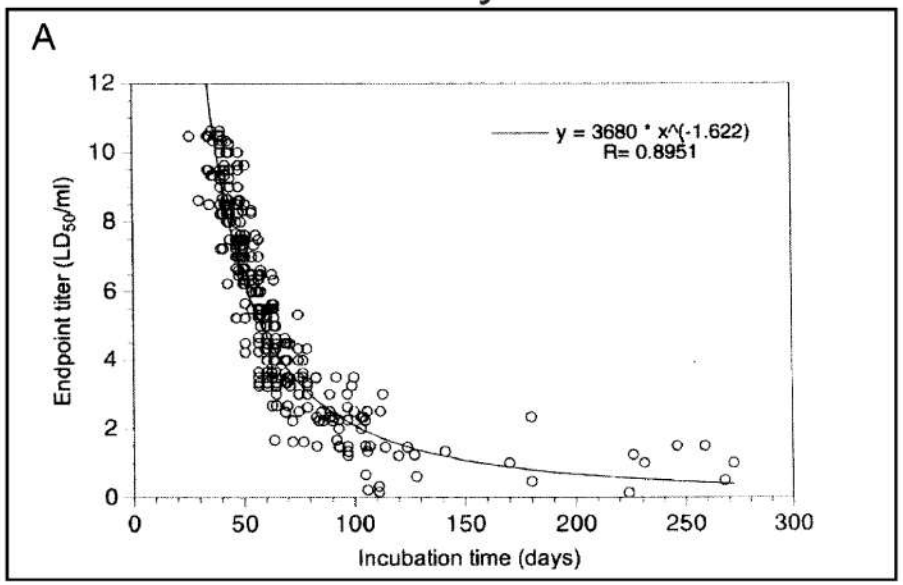
(Adapted from Prusiner 2004, Gajdusek et al. 1966)

Developing a model system to ID the ‘scrapie agent’

Endpoint titration assay



Incubation time assay



Initial findings:

The scrapie agent (whatever it is...) can replicate

(Adapted from Prusiner 2004)

Properties of the ‘scrapie agent’

Treatment	Viroid (PSTV)	Scrapie agent
Ionizing radiation	Relatively resistant It’s tiny! ~ 110 -137 kD	Relatively resistant It’s tiny! ~ 64 -150 kD
Nuclease treatment (RNase or DNase)	Sensitive	Resistant
UV radiation	Sensitive	Resistant →
Divalent cation hydrolysis	Sensitive	Resistant
Heat	Resistant (Most viruses sensitive)	Stable at 90° for 30 minutes
Urea	Resistant	Sensitive
Proteinase K	Resistant	It depends

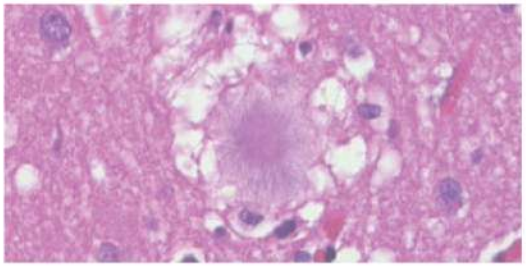
Table 3. Inactivation of small infectious agents by UV irradiation at 254 nm.

Example	D_{37} (J/m^2)*
Bacteriophage T2	4
Bacteriophage S13	20
Bacteriophage ϕX174	20
Rous sarcoma virus	150
Polyoma virus	240
Friend leukemia virus	500
Murine leukemia virus	1,400
Potato spindle tuber viroid	5,000
Scrapie agent	42,000

*Data from (*82*, *85*, *88*). D_{37} is the dose of irradiation that permits 37 percent survival.

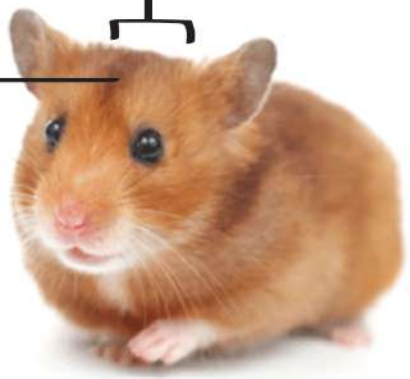
(Prusiner et al. 1982)

Purification of the scrapie agent ‘PrP’

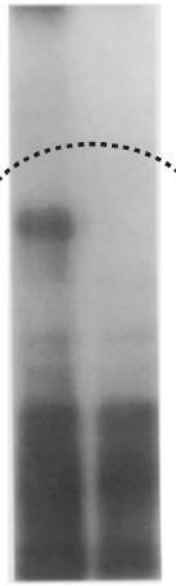


Intracerebral injection
of scrapie agent

~ 70 days

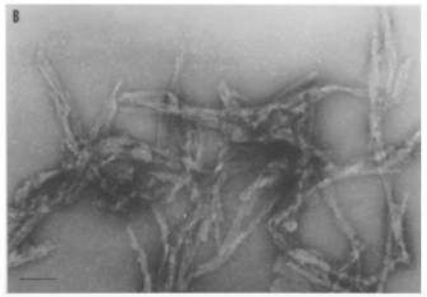


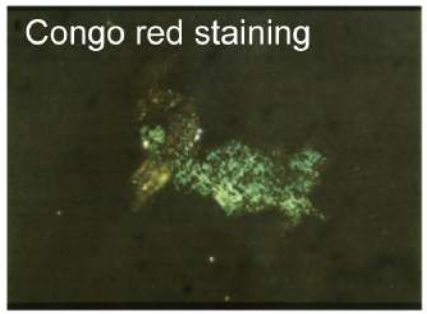
Purification



TEM

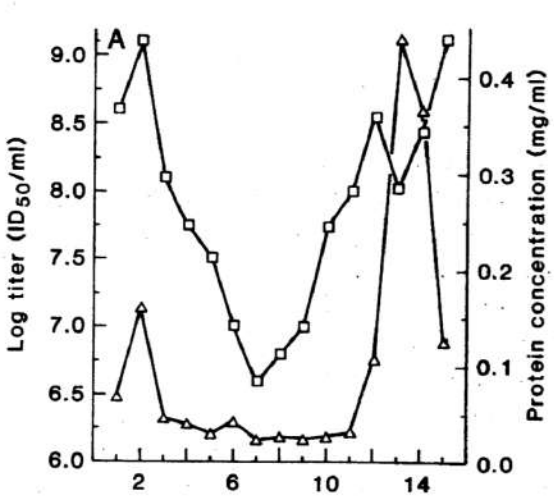
Fibrous ‘amyloid-like’ aggregates

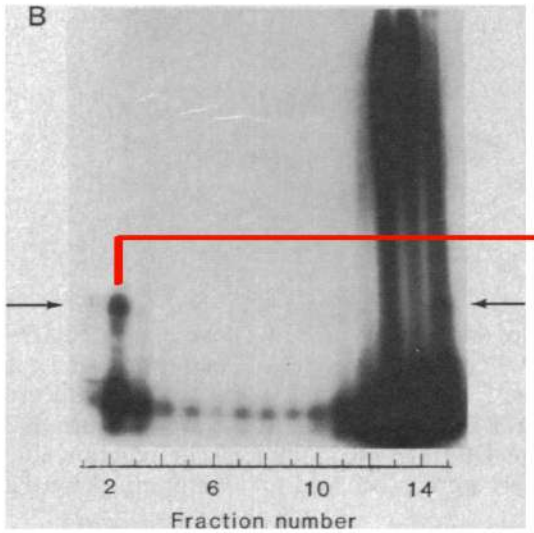




(McKinley et al., 1983, Prusiner et al., 1983, Oesch et al., 1984)

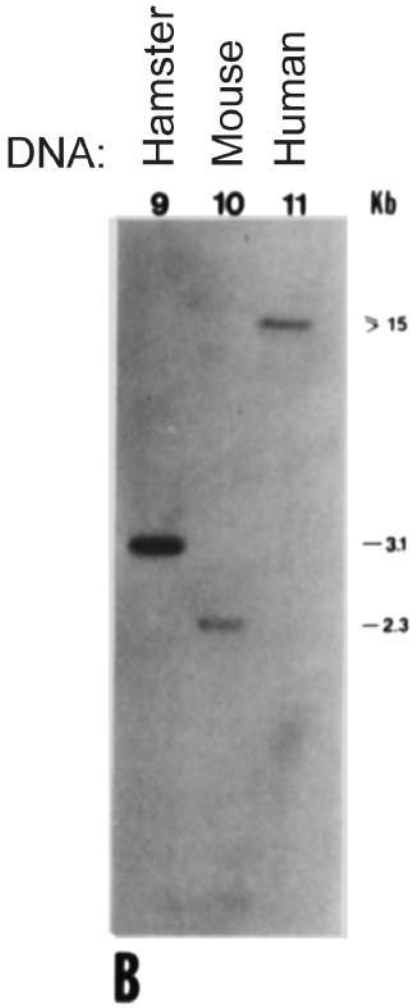
ID of the PRNP gene





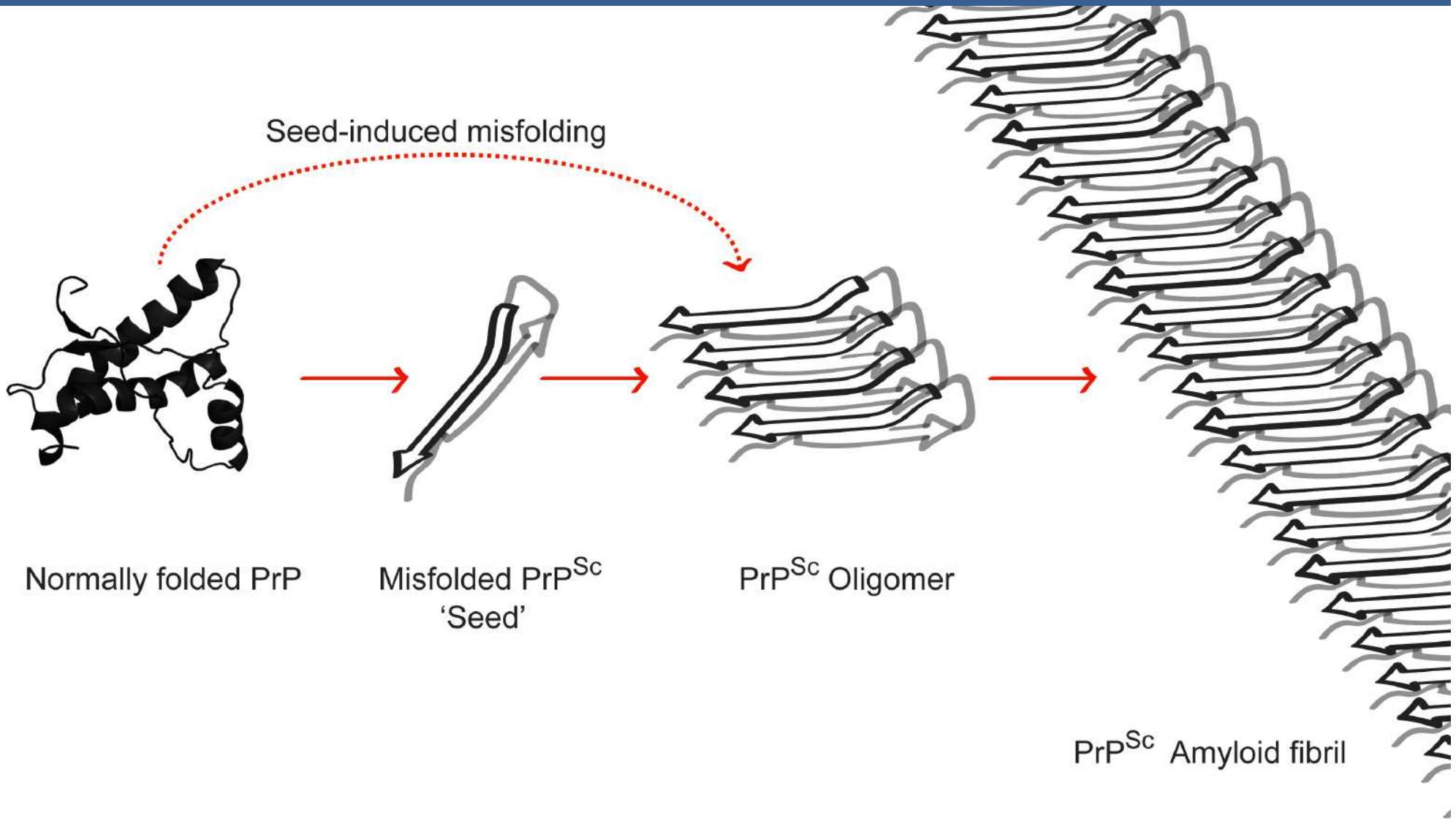
death about 2 weeks later. Poly(A)$^+$ RNA was isolated from scrapie-infected hamster brain 35 days after inoculation, and a cDNA library was prepared essentially by the procedure of Okayama and Berg (1982); however, a different vector construction was used (W. Boll and C. Weissmann, unpublished work). A set of 32 icosameric oligonucleotides, 5′-GG(T/C)TT(A/G)TTCCA(T/C)TG(A/G)TT(A/G)TG, was synthesized, based on the reverse translation of a seven amino acid segment of PrP 27-30, N-His-Asn-Gln-Trp-Asn-Lys-Pro-C (Prusiner et al., 1984). Screening of 150,000 colonies with the 5′-[^{32}P]-labeled probe mixture yielded a positive clone, from which the recombinant plasmid pHaPrP was isolated.

Sequence PrP (aa)



(Prusiner et al. 1982, Oesch et al. 1985)

The prion hypothesis



TSE ‘transmission barrier’

Prion-contaminated meat

Cow → Prion-contaminated meat → Humans

BSE
‘Mad Cow Disease’

‘Variant CJD’

Scrapie inoculum

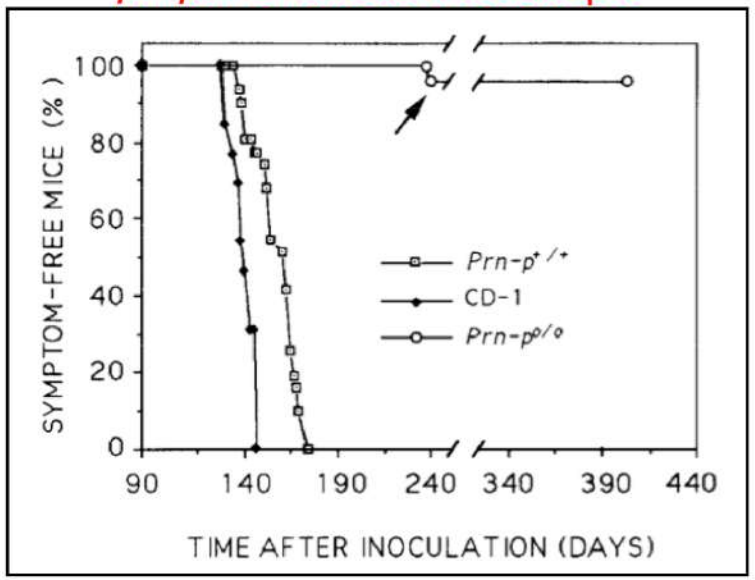
Mice
~18 months
~4-5 months

Hamster
~70 days

Tg Mice
>70 days

Prions composed of mouse/hamster

*prnp*Δ is resistant to scrapie

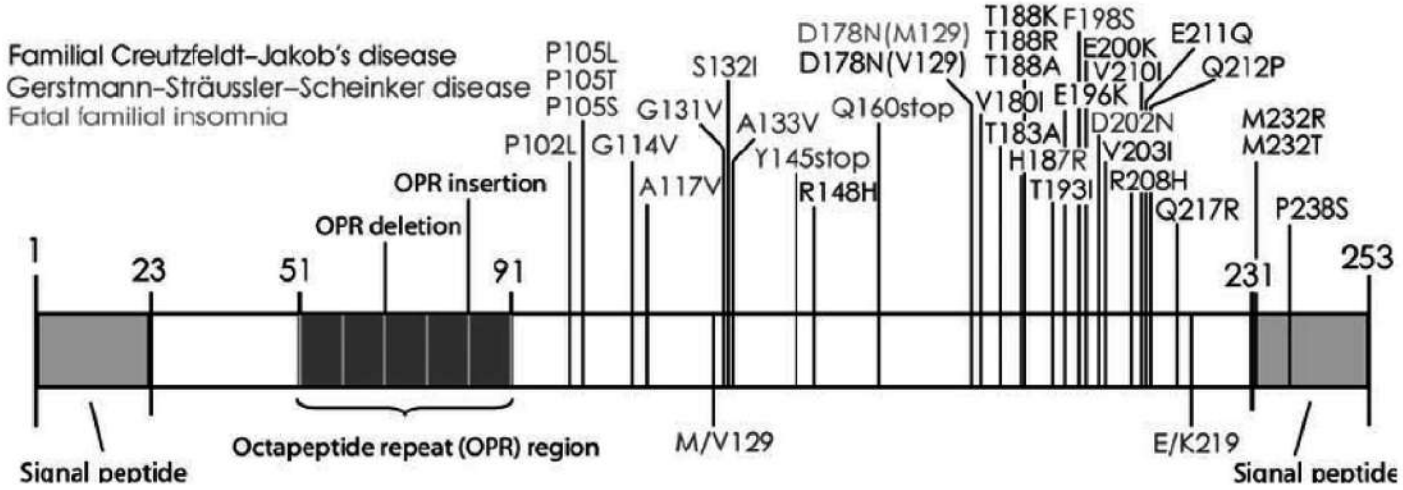


(Bueler et al. 1993)

An alternate path to discovery

Disease	Mechanism of pathogenesis
Kuru	Infection through ritualistic cannibalism
Iatrogenic Creutzfeldt-Jakob Disease	Infection prion-contaminated medical instruments/ cornea transplants etc.
Variant CJD	Consumption of Bovine prions
Familial CJD	?
Fatal Familial Insomnia	?
Gerstmann-Straussler-Scheinker disease	?

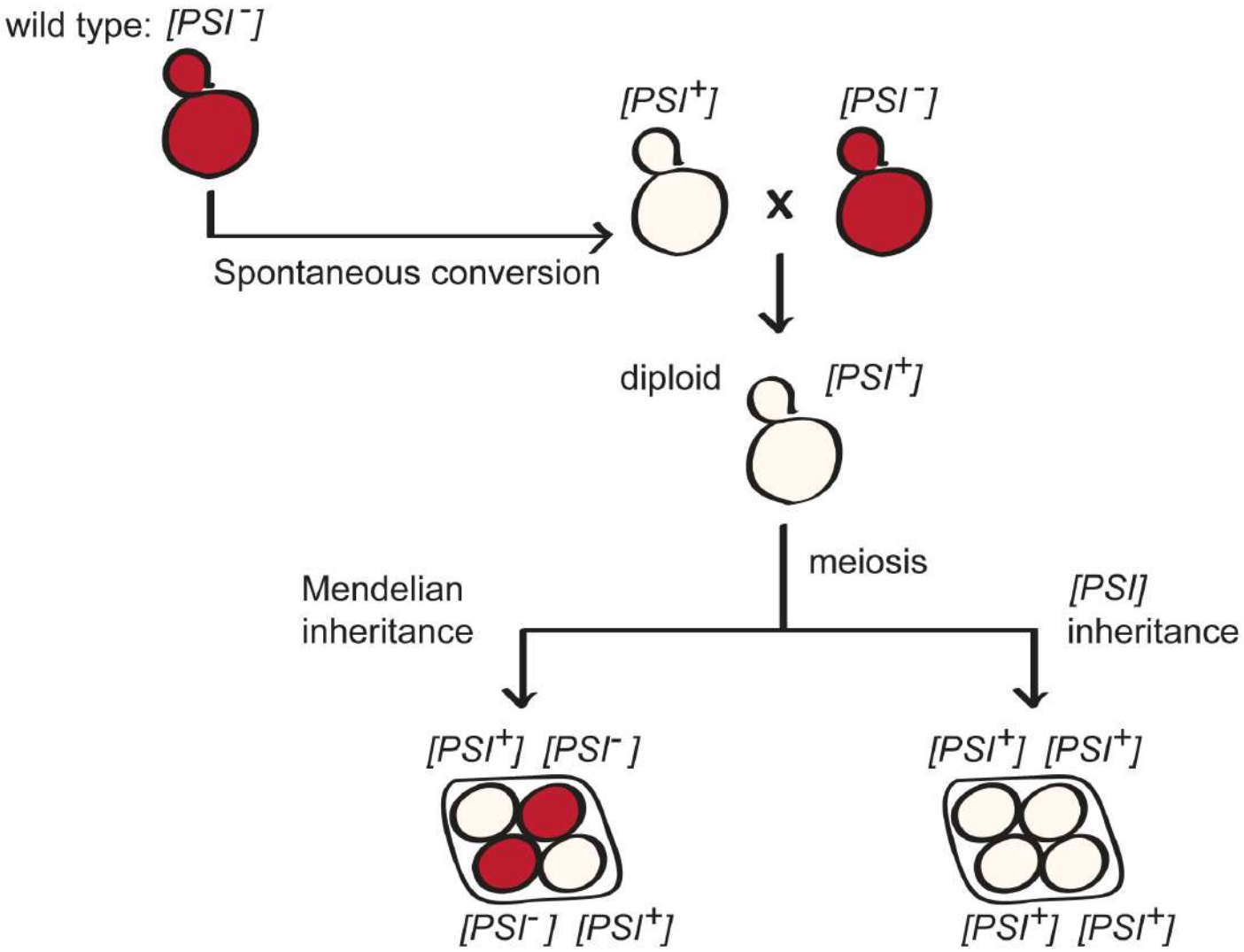
PRNP gene



(Adapted from Prusiner 2004, van der Kamp and Daggett 2009)

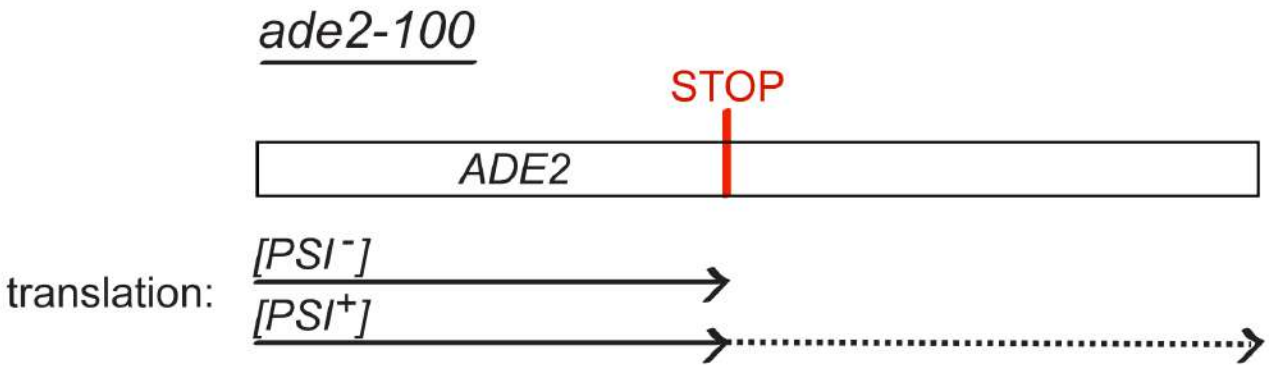
Fungal prions

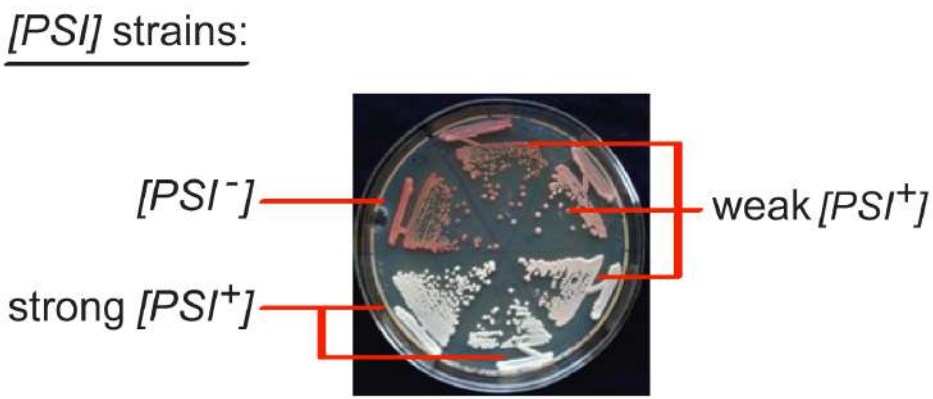
The strange inheritance pattern of the genetic element [*PSI*]



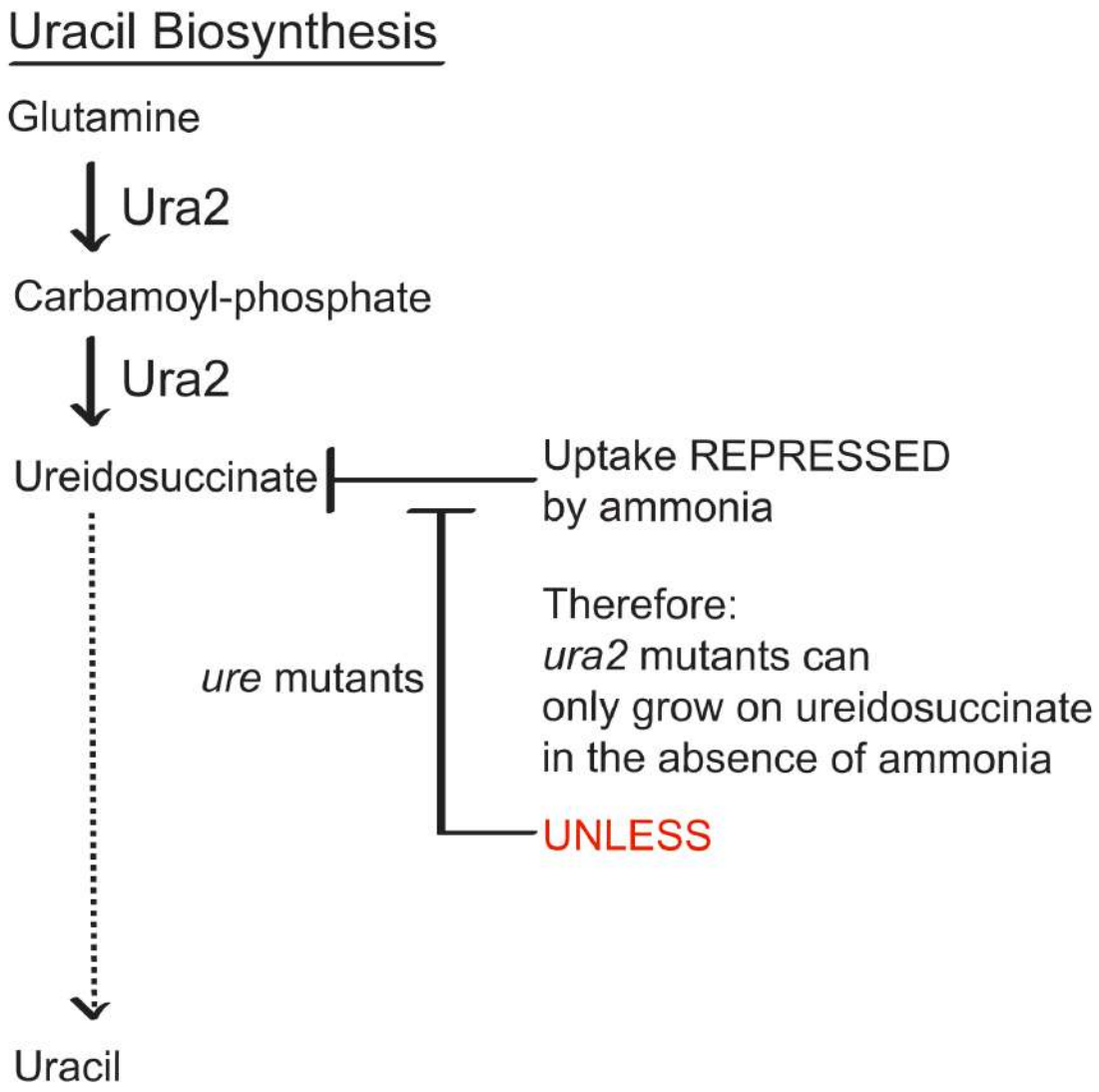
(Cox 1965)

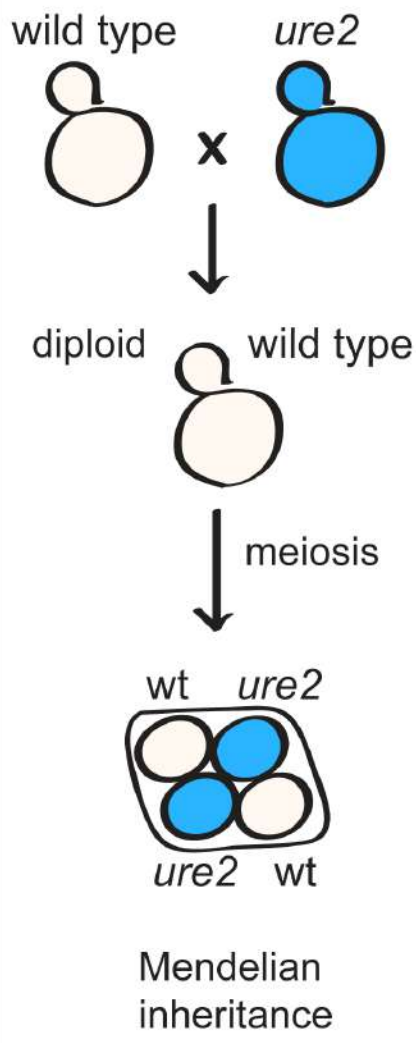
[*PSI*$^{+}$] causes stop codon read-through

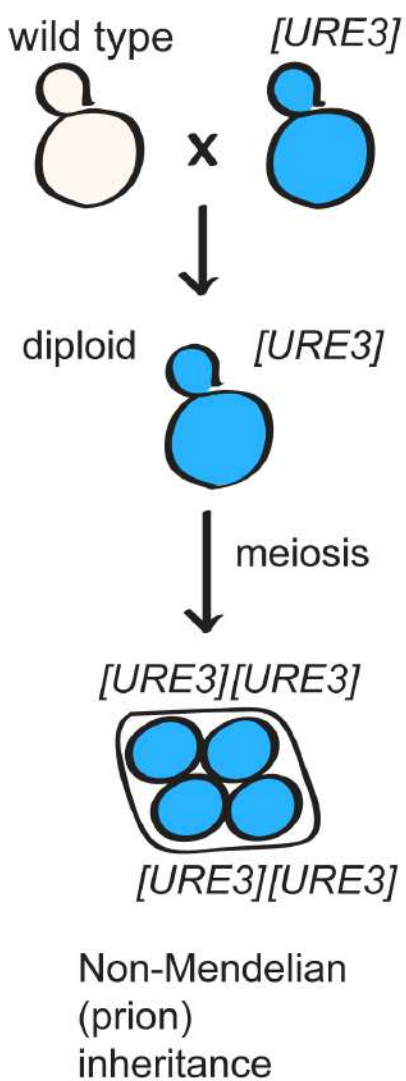




[*URE3*] is a non-Mendelian genetic element





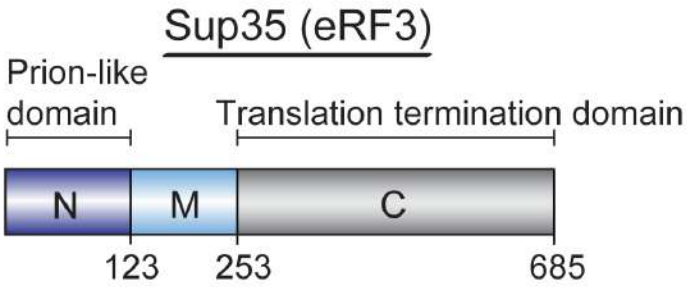


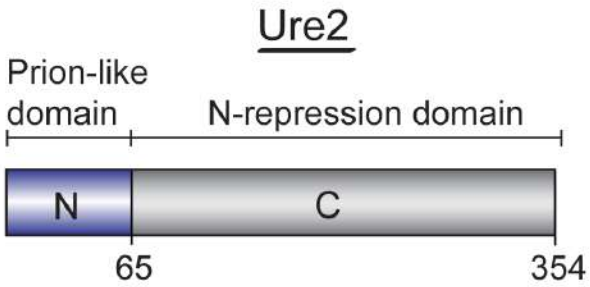
[*PSI*] and [*URE3*] are caused by prion forms of Sup35 and Ure2

- Induced by overexpression of *SUP35* and *URE2*
- Require functional *SUP35* and *URE2* coding regions to propagate

---

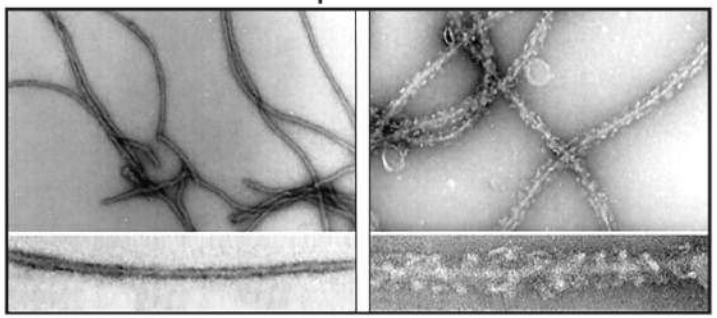
- "Functional" and "prion-like" domains are separable



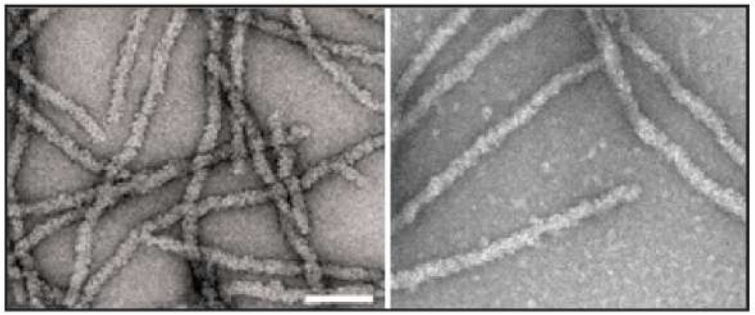


- Purified protein forms self-seeded fibrils

Recombinant Sup35

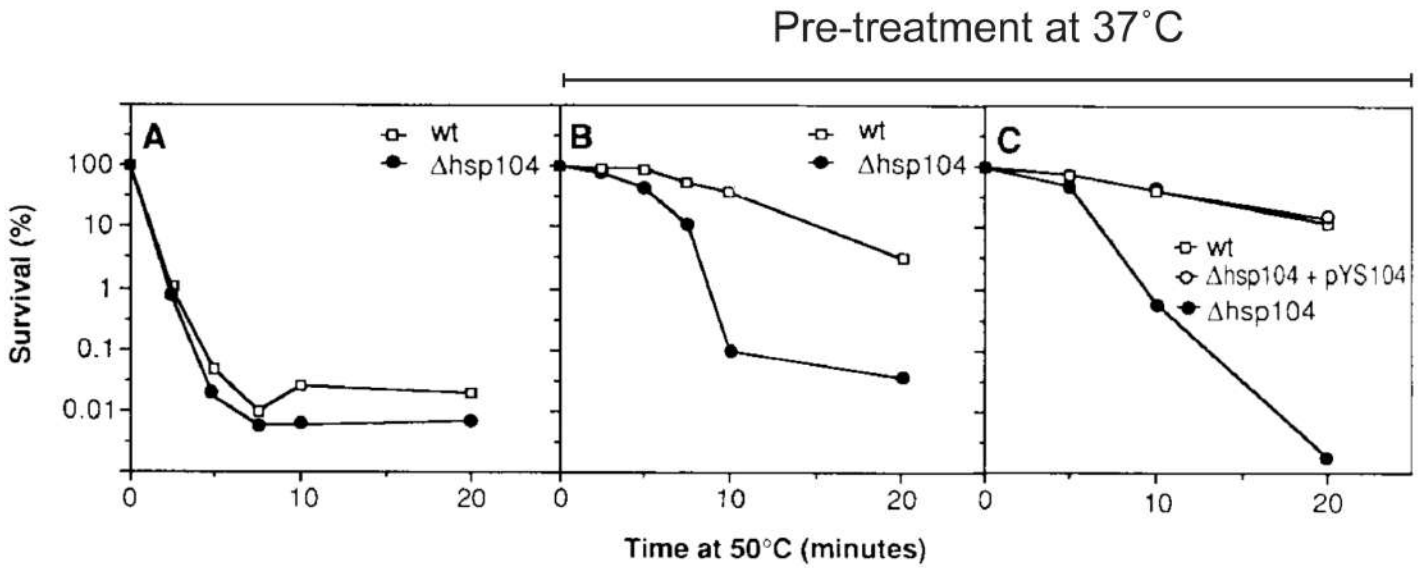


Recombinant Ure2

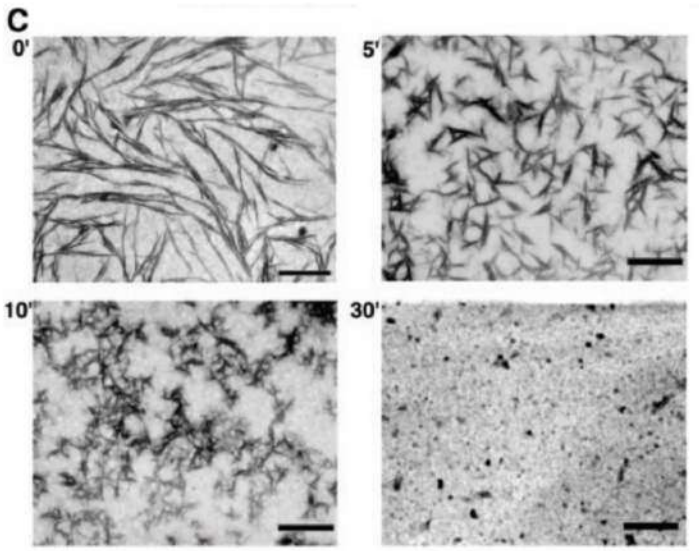


(Masison and Wickner 1995, Glover et al. 1997, Taylor et al. 1999)

Chaperones influence prion formation and propagation

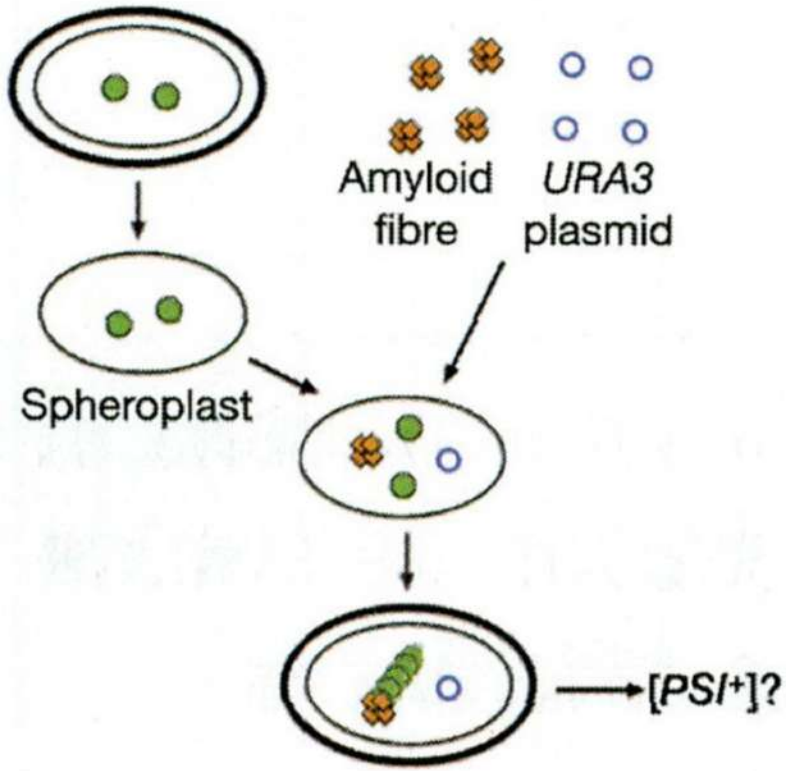


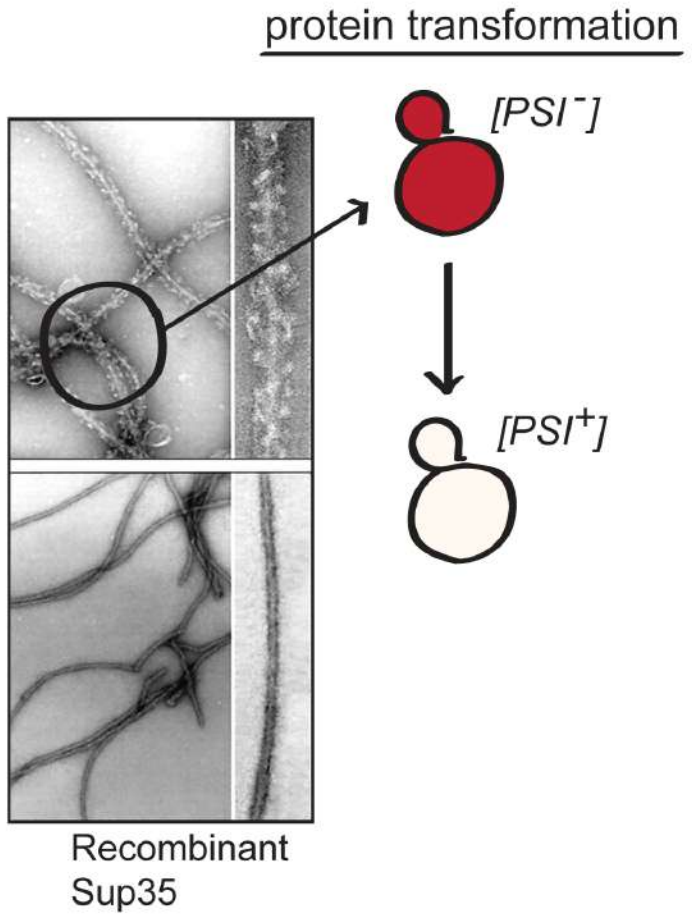
- *hsp104*Δ cannot propagate [*PSI*$^+$]
- Overexpression of *HSP104* can cure [*PSI*$^+$]
- Hsp104 protein can dissassemble Sup35 fibers in vitro



(Sanchez and Lindquist 1990, Shorter and Lindquist 2004)

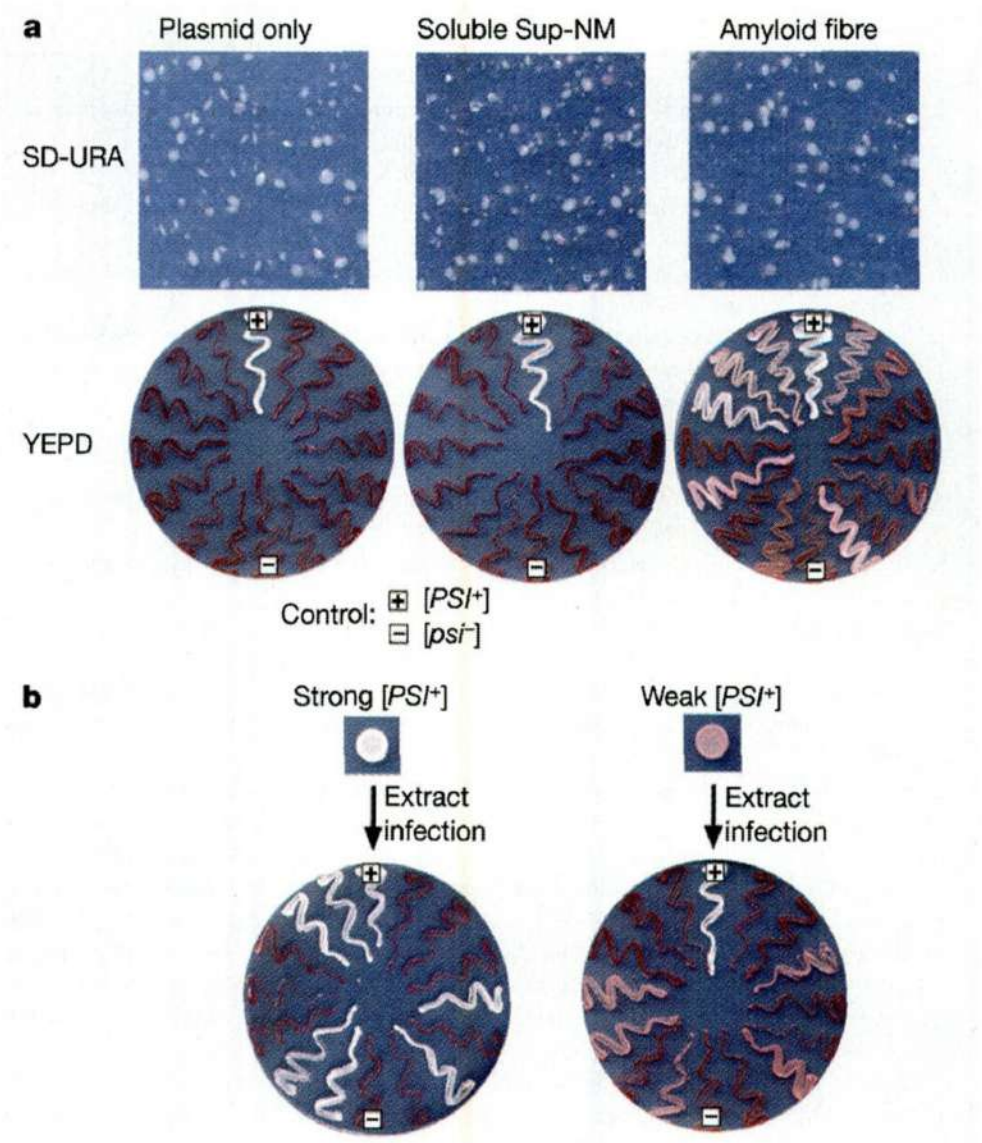
Transformation of Sup35 amyloids into yeast





(Tanaka et al. 2004)

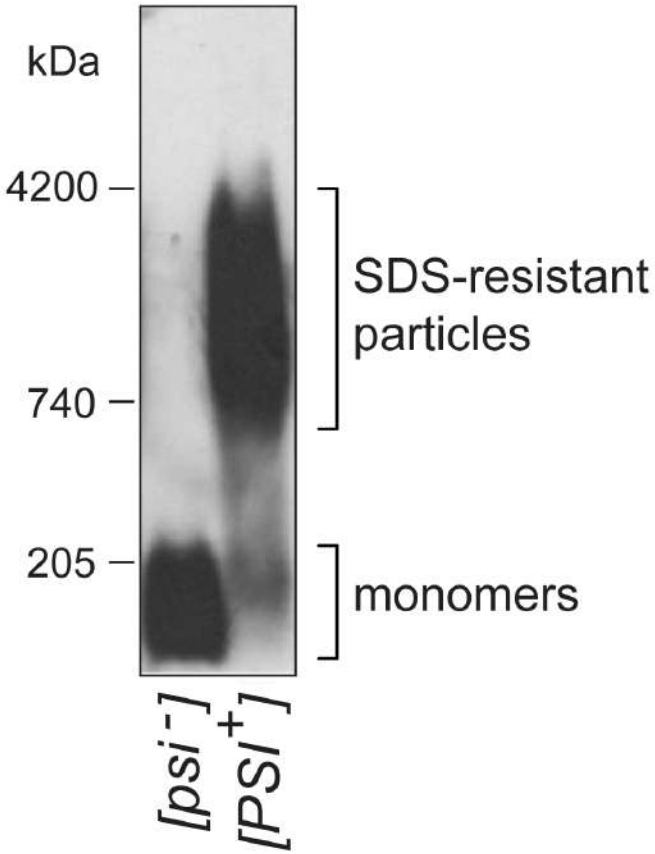
Prion strains result from different seeds



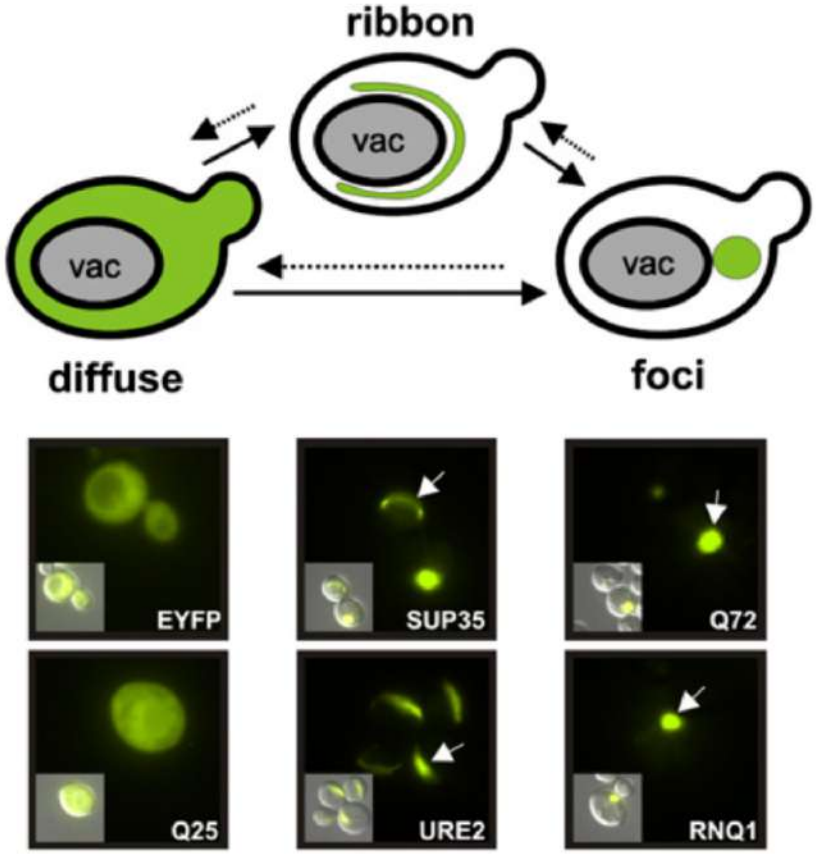
(Tanaka et al. 2004)

Other methods to study prions

SDD-AGE

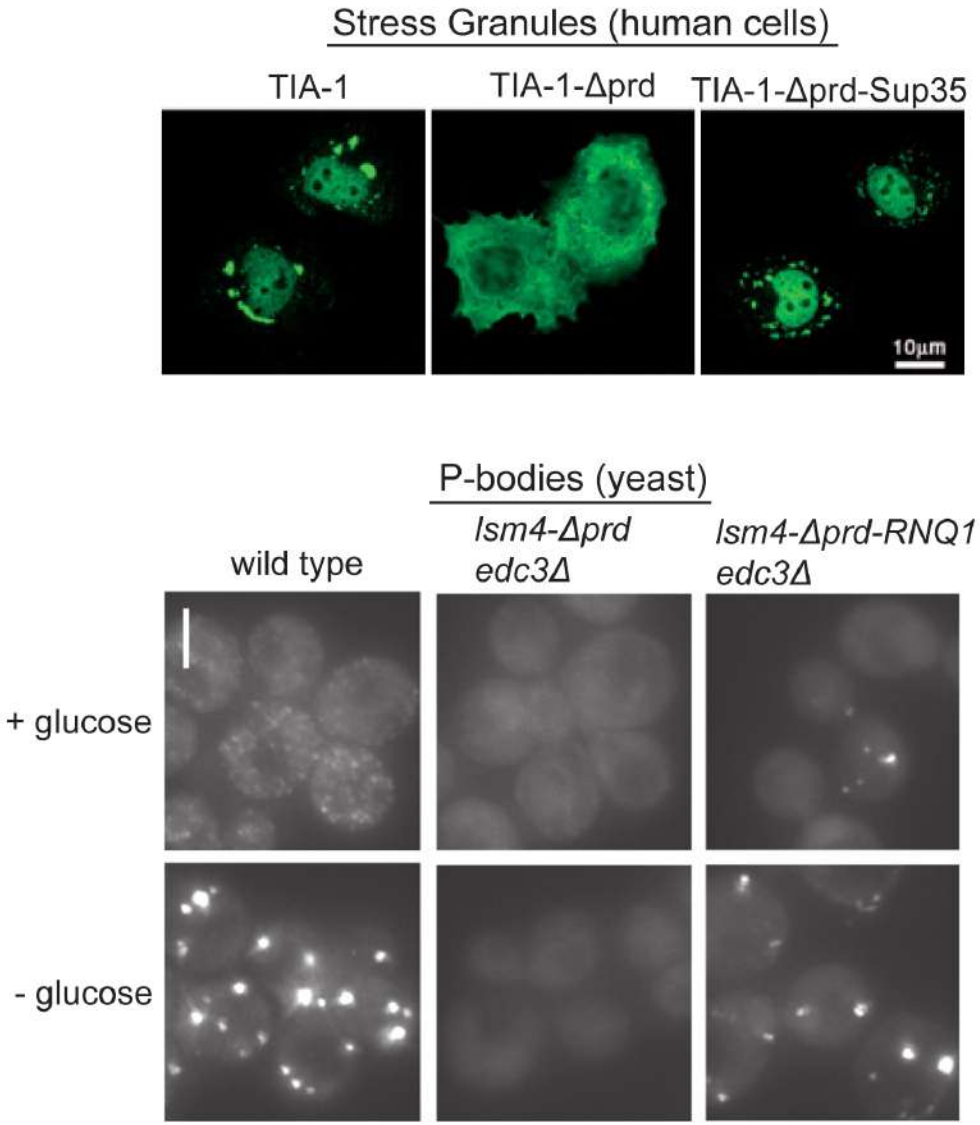


Fluorscence microscopy



(Kryndushkin et al. 2003, Alberti et al. 2009)

Prion-like domains are often modular and portable



(Gilks et al., 2004, Decker et al., 2007)

Computational methods to ID prions

- Search for N/Q-rich stretches (and biases towards G, S, and/or Y)
  - Michelitsch and Weissman, 2000
  - Harrison and Gerstein, 2003

- Algorithm designed to ID similarity to known 'prion-like' regions
  - Alberti et al. 2009

- Scoring amino acid sequences based on experimentally-derived prion propensities from scrambled sequences
  - Toombs et al. 2010

So what is a prion?

Prions are *transmissible* protein conformers that *self-replicate* via *templating* the conversion of other copies of the same protein and promote *phenotypic change*.

1) They behave as non-Mendelian genetic elements.

2) The associated phenotype will be reversible.
"Curability"

3) A maintentance gene encoding the normal protein will manifest as a related, Mendelian genetic element.

4) Overproduction of the maintenance element gene product will increase the generation of the non-Mendelian element.
- "Seeding"

5*) Transformation of prionized protein particles will cause the associated prion phenotype.

Unanswered Questions

PrP and TSE's:

- What are the factors required to trasmit PrP^{Sc}?
- What is the function of PrP?

General prion-related phenomena:

- To what extent and how are prions and amyloid-related diseases connected?
- What is the link between RNA biology and prions?
- Are prions diseases, beneficial, or both?
- How do evolutionary pressures influence prions and vice versa?
- What are the sequence and environmental factors that specify prions?