YEAST MODEL FOR STUDYING HERITABLE MAMMALIAN PRION DISEASES

Yury O. Chernoff

School of Biology,
Institute for Bioengineering and Bioscience,
Center for Nanobiology of the
Macromolecular Assembly Disorders
(NanoMAD),
Georgia Institute of Technology,
Atlanta

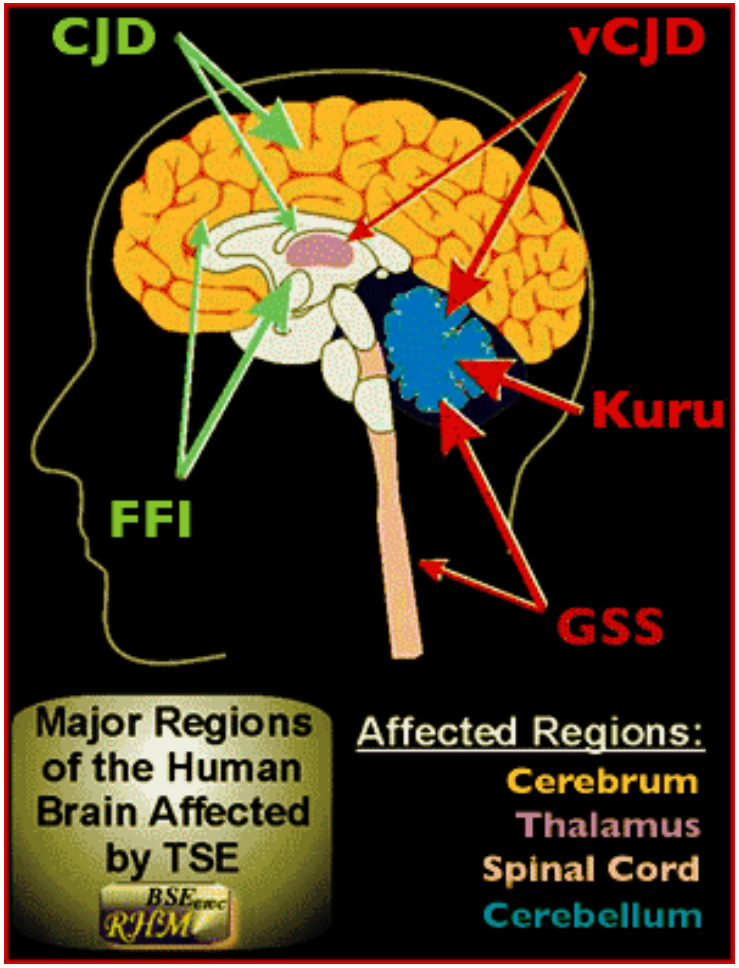
PRION DISEASES

HUMAN PRION DISEASES:

- **Creutzfeldt-Jakob disease (CJD)**
- **Gerstmann-Straussler-Scheinker syndrome (GSS)**
- **Fatal familial insomnia (FFI)**
- **Kuru**
  (perpetuated by cannibalism among Fore people in Papua / New Guinea)

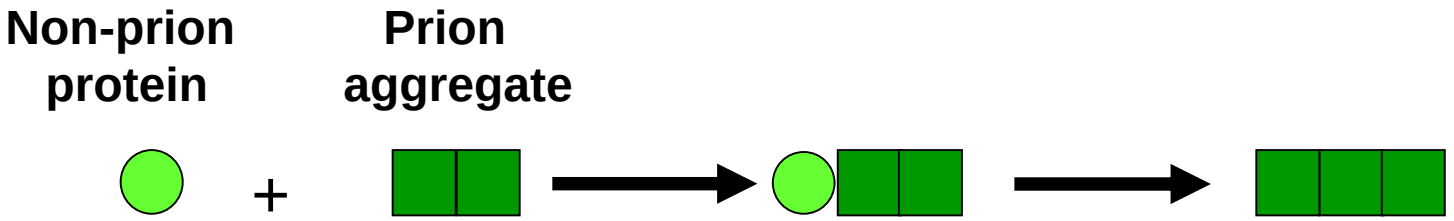
ALSO FOUND IN OTHER MAMMALS
(Sheep scrapie, "mad cow" disease, *etc*.)

PRION DISEASES ARE INCURABLE AND CAUSE ULTIMATE DEATH



"MAD COW" DISEASE IS TRANSMISSIBLE TO HUMANS AND CAUSES "VARIANT CJD (vCJD)"

PRION MODEL



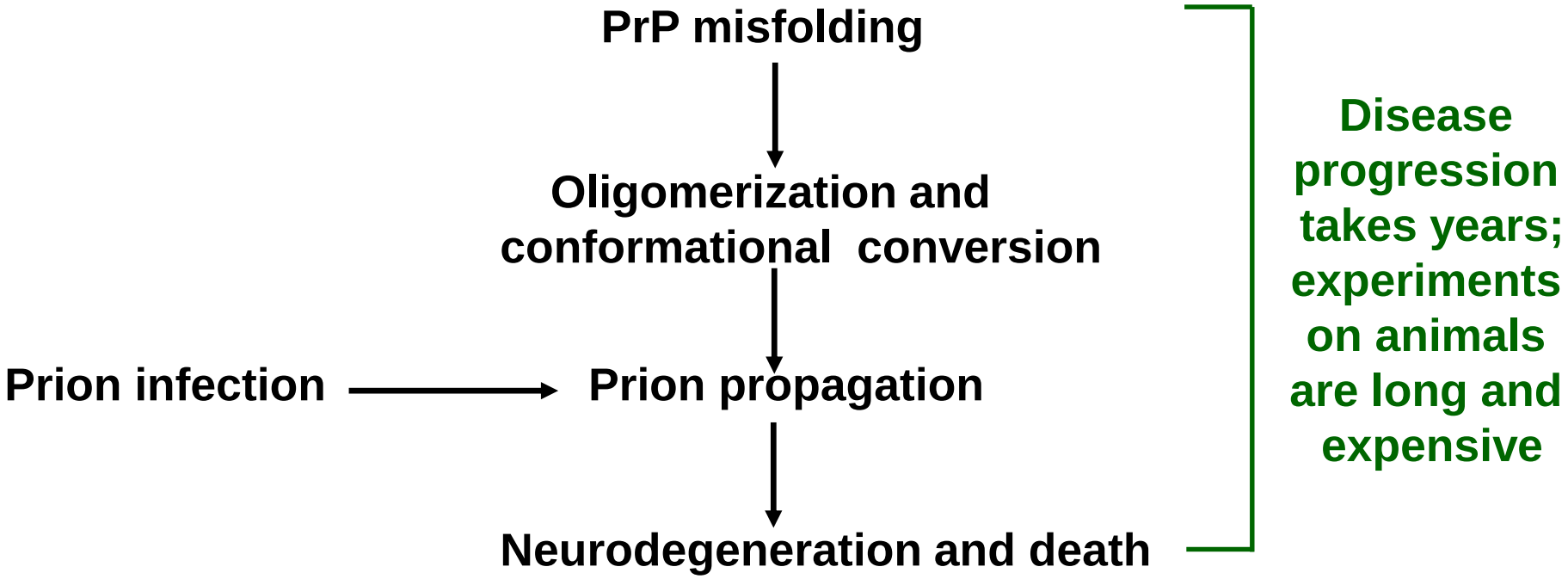
- **Prions produce self-perpetuating protein aggregates (amyloids)**
- **Prion form of the specific protein (PrP in mammals and humans) can convert a non-prion protein of the same amino acid sequence into a prion form**
- **Prion pathology is reminiscent of other amyloidoses and neural inclusion diseases (Alzheimer's disease, Parkinson's disease, Huntington's diseases, etc.)**

Prions are like "molecular tumors" that can spread to other cells/organisms

TYPES OF PRION DISEASES

- **Infectious** – transmitted by prion agent (PrP protein in a prion form)
- **Heritable –** caused by mutations in the PrP-coding gene
- **Sporadic –** occur spontaneously, initial cause is unknown

PATHWAY OF DISEASE DEVELOPMENT



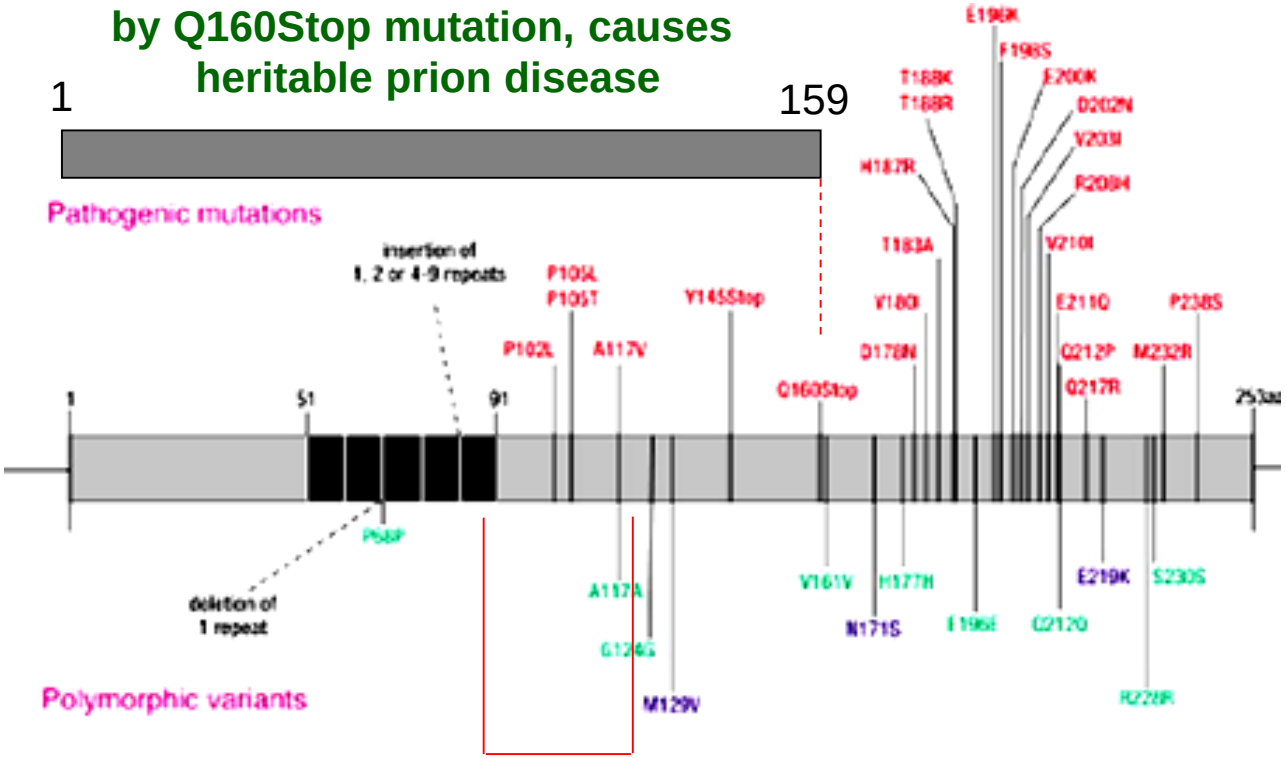
Only treatments targeting early steps could eliminate cause of the disease

However, it is hard to determine in mammalian models which step is targeted by a drug

HERITABLE PRION DISEASES
(15% of all cases in humans)

ORIGINATE FROM MUTATIONS IN THE GENE CODING FOR PrP PROTEIN

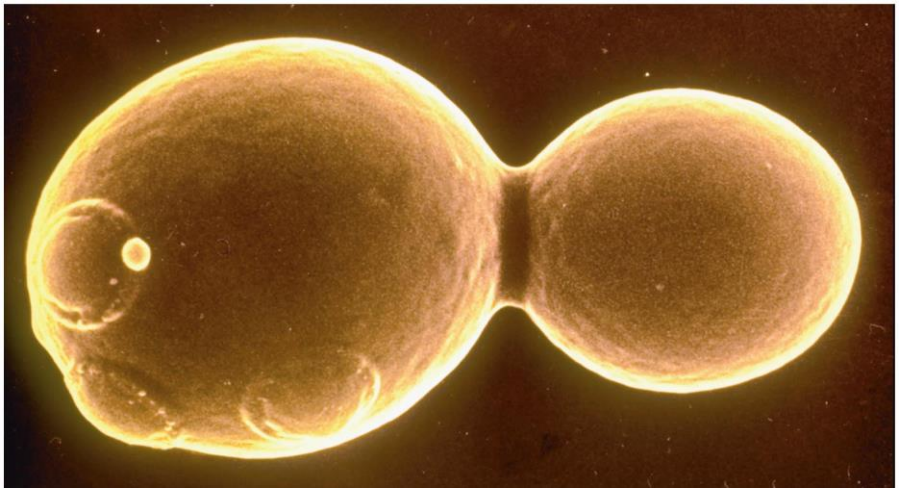
Shortened protein, generated by Q160Stop mutation, causes heritable prion disease



Region 90-120 is required for prion disease

PROBLEM:
It remains unclear which stage of prion pathway is influenced by each mutation

YEAST AS A MODEL ORGANISM



Yeast *Saccharomyces cerevisiae* is a unicellular microscopic fungus

Organization of the cell is similar to animals and humans

Is cheap and easy to cultivate in large quantities, and experiments can be performed fast

Was used successfully as a model for understanding the mechanism of human diseases, including various forms of cancer

CAN YEAST MODEL BE APPLIED TO PRION STUDIES?

YEAST PRIONS

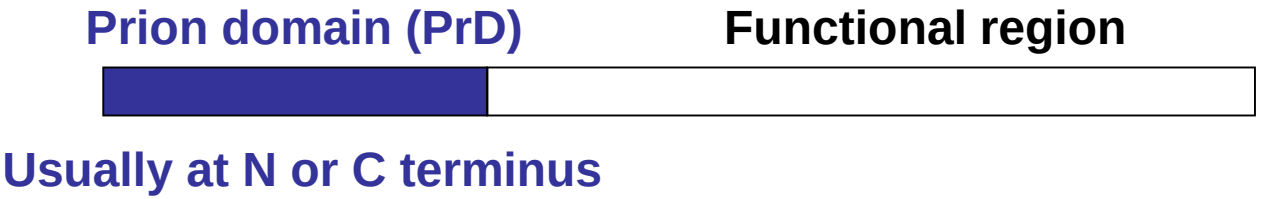
Amyloids transmitted via cytoplasm

At least 9 different proteins are proven to behave as prions in yeast

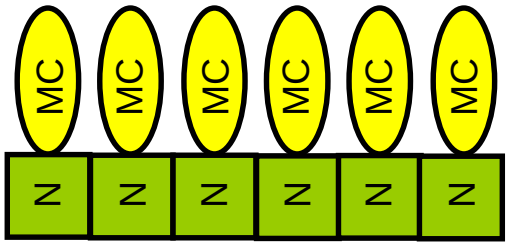
More than 20 proteins contain domains with proven prion properties
More than 100 prion candidates in the yeast genome

Some prions are pathogenic to yeast and some are not

Yeast prions are homologous to neither mammalian PrP nor each other



YEAST PRION PROTEIN SUP35

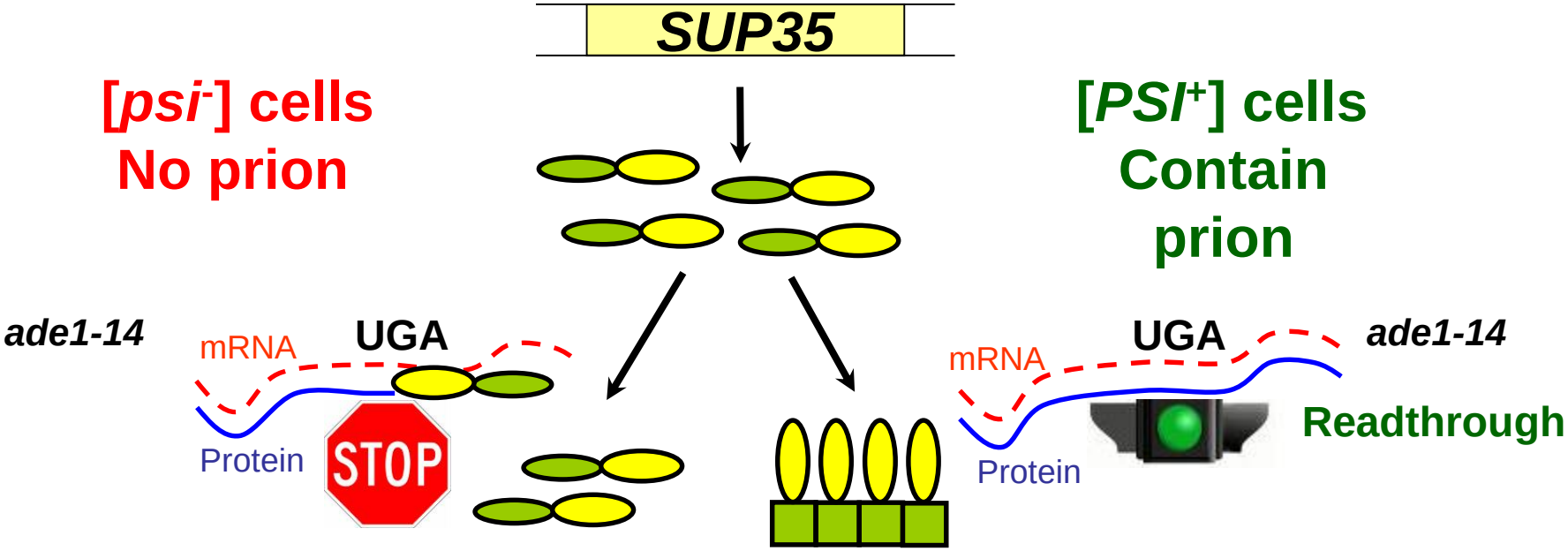


Middle (M) and functional (C) domains

Prion domain (N)

- Sup35N (prion domain) forms the core of the fiber
- Sup35C (and possibly M) domains are exposed on the side
- Ends of the fiber are active sites for immobilization of new Sup35 molecules

READTHROUGH ASSAY DETECTS SUP35 PRION ([*PSI*$^+$]) IN YEAST



Truncated Ade1 protein

No growth on –Ade

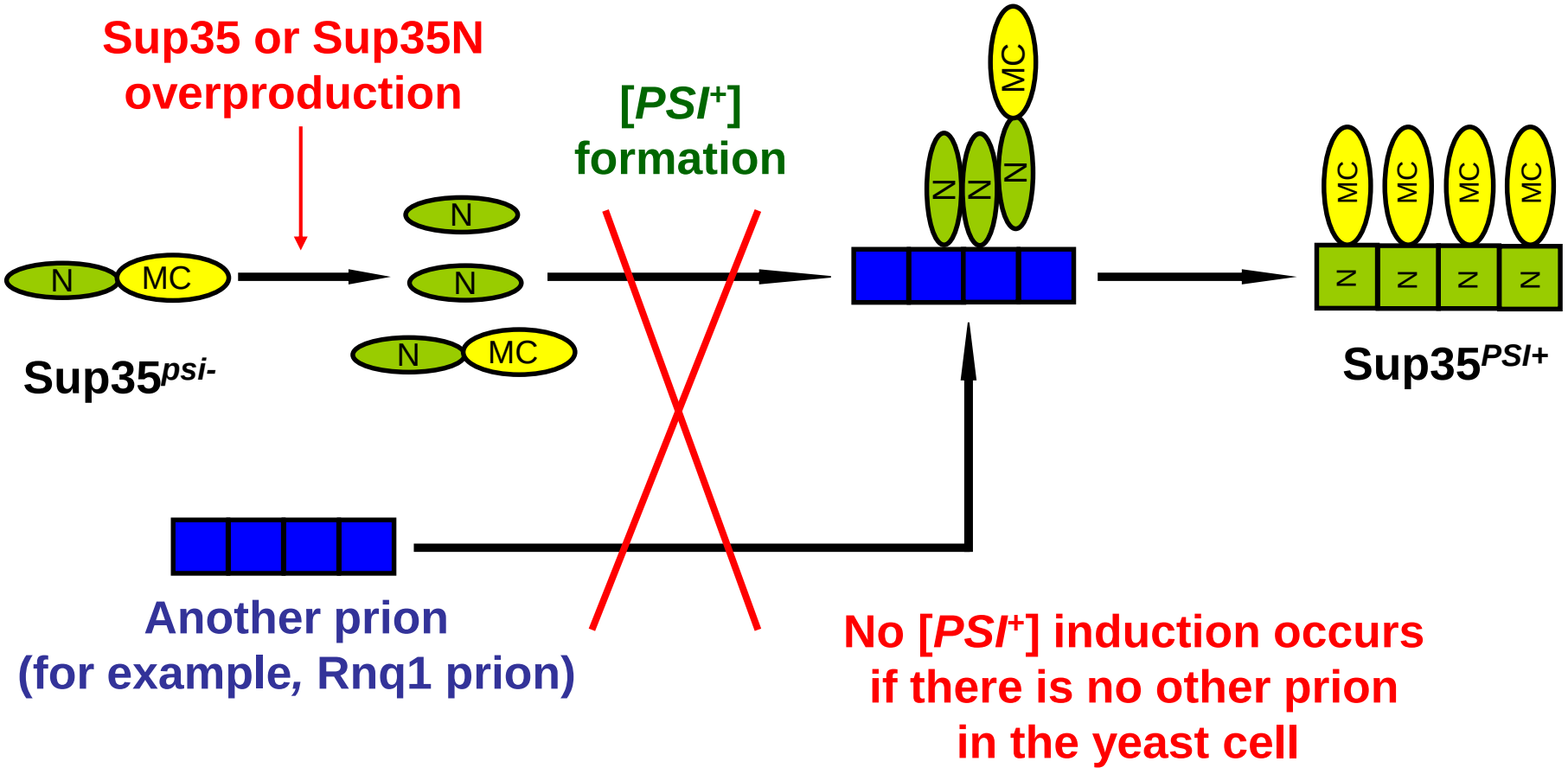
Red on YPD

Full-length Ade1 protein

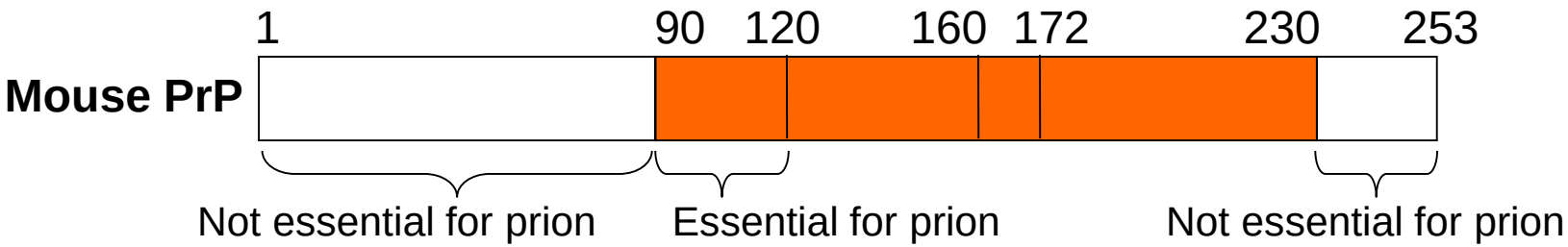
Growth on –Ade

White on YPD

FORMATION OF THE [*PSI*$^{+}$] PRION DEPENDS ON PROTEIN LEVELS AND ON OTHER PRIONS



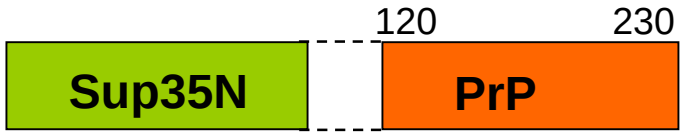
FUSION OF SUP35N TO PrP INDUCES [*PSI*$^{+}$] IN THE ABSENCE OF OTHER PRIONS



MAMMALS / HUMANS

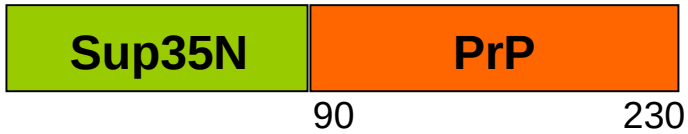
YEAST

Not susceptible to prion disease



No prion induction

Susceptible to prion disease



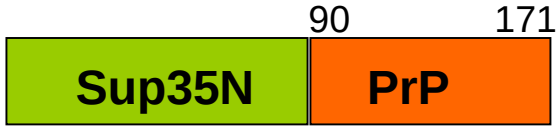
Prion induction

Not applicable

No prion induction

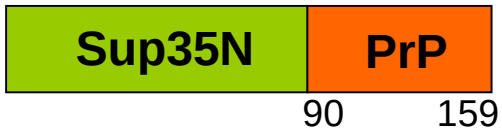
Not applicable

Not tested (expected to cause a disease from our data)



Strong induction

Causes heritable prion disease

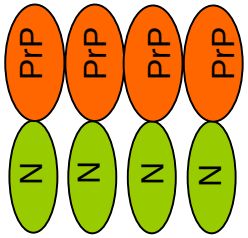


Strong induction

MODEL OF PRION INDUCTION BY SUP35N-PrP IN YEAST

Prion properties of PrP drive formation of the yeast prion

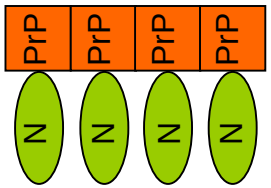
Oligomer formation by PrP domains



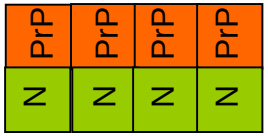
Needs 90-120 region

Oligomer stabilization due to conformational conversion?

Counteracted by PrP C-terminus, stabilizing the native conformation

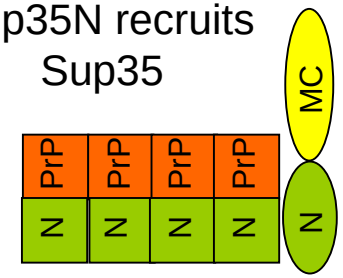


Sup35N converts into a prion state

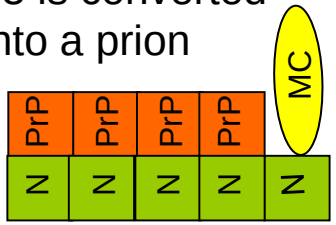


Oligomerization and conformational conversion steps are being specifically targeted

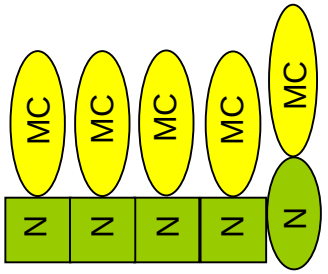
Sup35N recruits Sup35



Sup35N domain of Sup35 is converted into a prion



Prion propagation



FUTURE PLANS

To characterize other known mutations, associated with heritable prion disease in mammals, in the yeast model, and to determine the stages in prion pathway, affected by these mutants

To perform systematic mutational analysis of PrP in the yeast system in order to determine which amino acid residues are crucial for prion formation

Based on this information, search for anti-prion treatments (for example, altered PrP derivatives or peptides) by using yeast

CONCLUSIONS

- **We have developed a yeast detection assay based on the ability of mammalian prion protein (PrP) to promotes prion formation by a yeast protein**
- **Prion properties of mammalian PrP in yeast are controlled by the same regions that control heritable prion disease in mammals and humans**
- **Yeast model can be employed for understanding the initial stages of prion formation and identifying the antiprion treatments**

ACKNOWLEDGEMENTS

Andrey Romanyuk

Pavithra Chandramowlishwaran

Meng Sun

Collaborators:
Carmen Krammer
(now at Northwestern University, USA)
Ina Vorberg (Bonn, Germany)

We are grateful to The CJD Foundation for financial support