

YEAST MODEL FOR STUDYING HERITABLE MAMMALIAN PRION DISEASES

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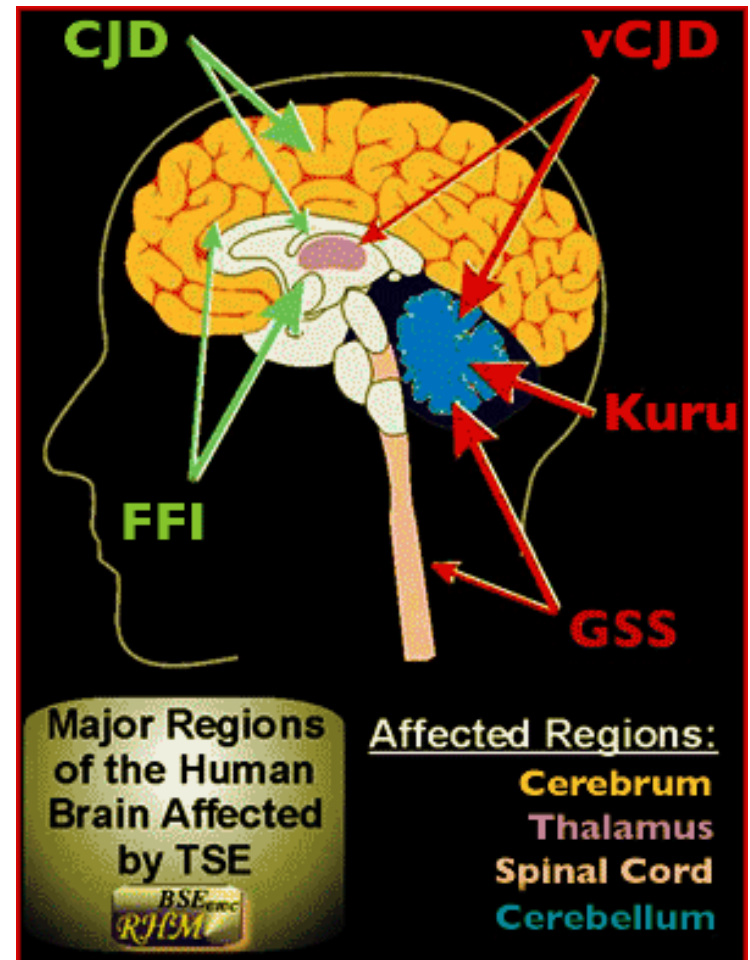
PRION DISEASES

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)

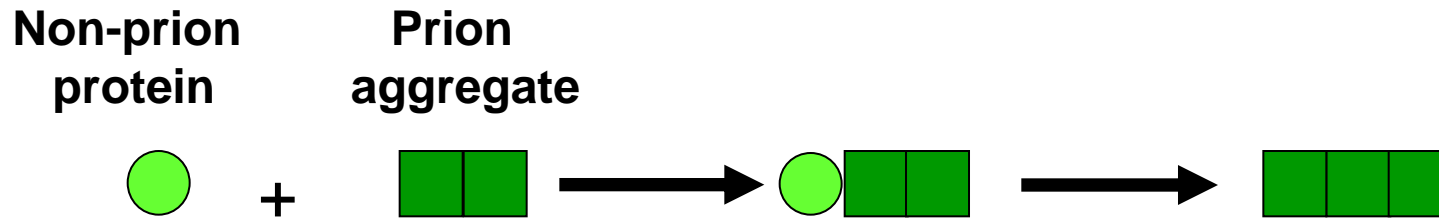
ALSO FOUND IN OTHER MAMMALS
(Sheep scrapie, “mad cow” disease, etc.)

**PRION DISEASES ARE INCURABLE
AND CAUSE ULTIMATE DEATH**



**“MAD COW” DISEASE IS
TRANSMISSIBLE TO
HUMANS AND CAUSES
“VARIANT CJD (vCJD)”**

PRION MODEL



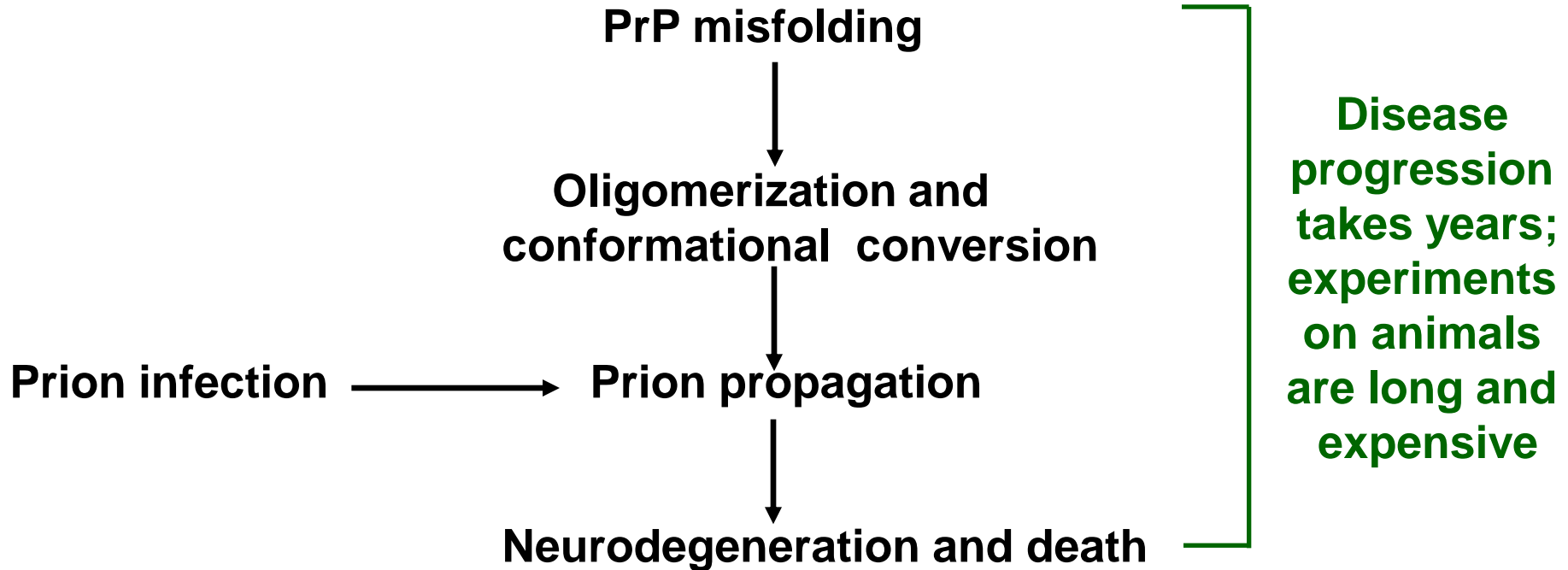
- Prions produce self-perpetuating protein aggregates (**amyloids**)
- Prion form of the specific protein (**PrP** in mammals and humans) can convert a non-prion protein of the same amino acid sequence into a prion form
- Prion pathology is reminiscent of other amyloidoses and neural inclusion diseases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

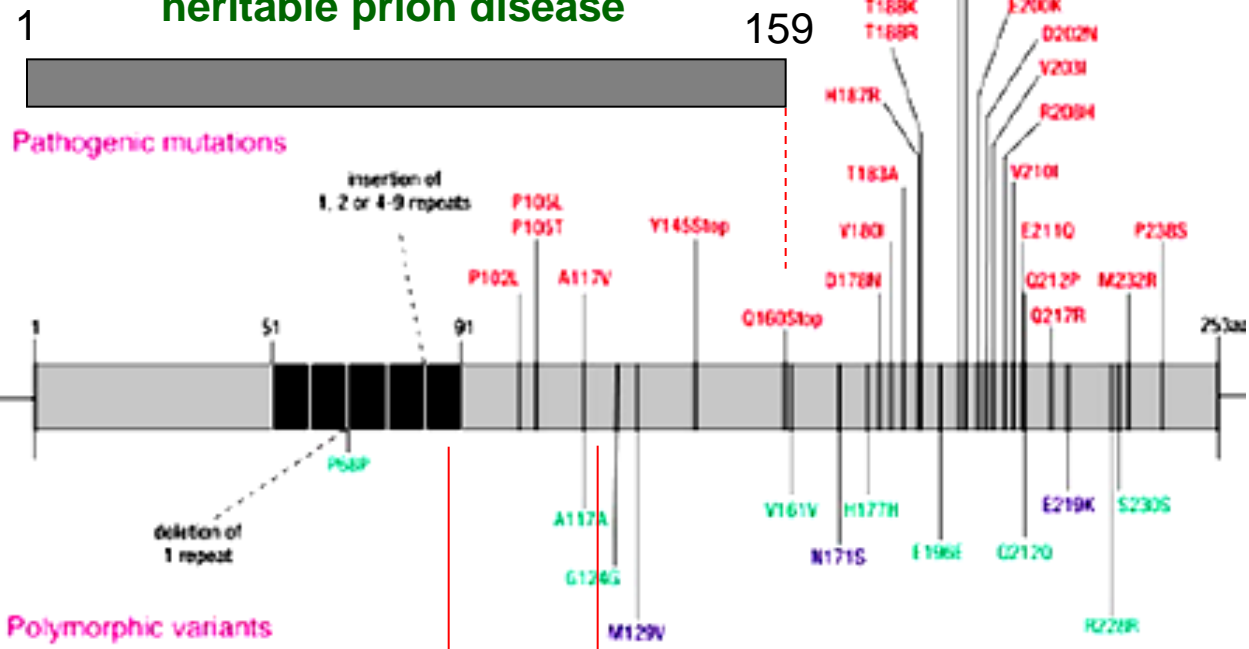
However, it is hard to determine in mammalian models which step is targeted by a drug

HERITABLE PRION DISEASES

(15% of all cases in humans)

ORIGINATE FROM MUTATIONS IN THE GENE CODING FOR PrP PROTEIN

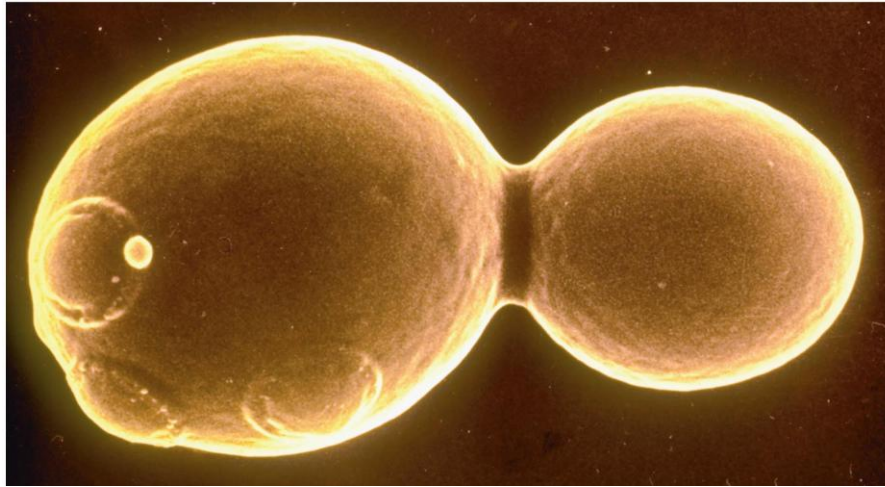
Shortened protein, generated by Q160Stop mutation, causes heritable prion disease



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

Region 90-120 is required for prion disease

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

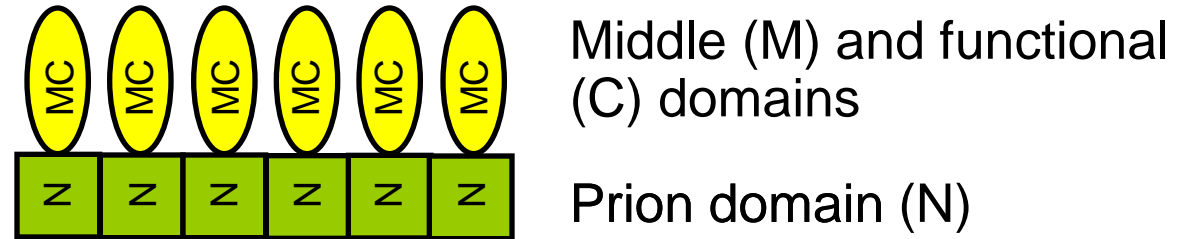
Prion domain (PrD)

Functional region



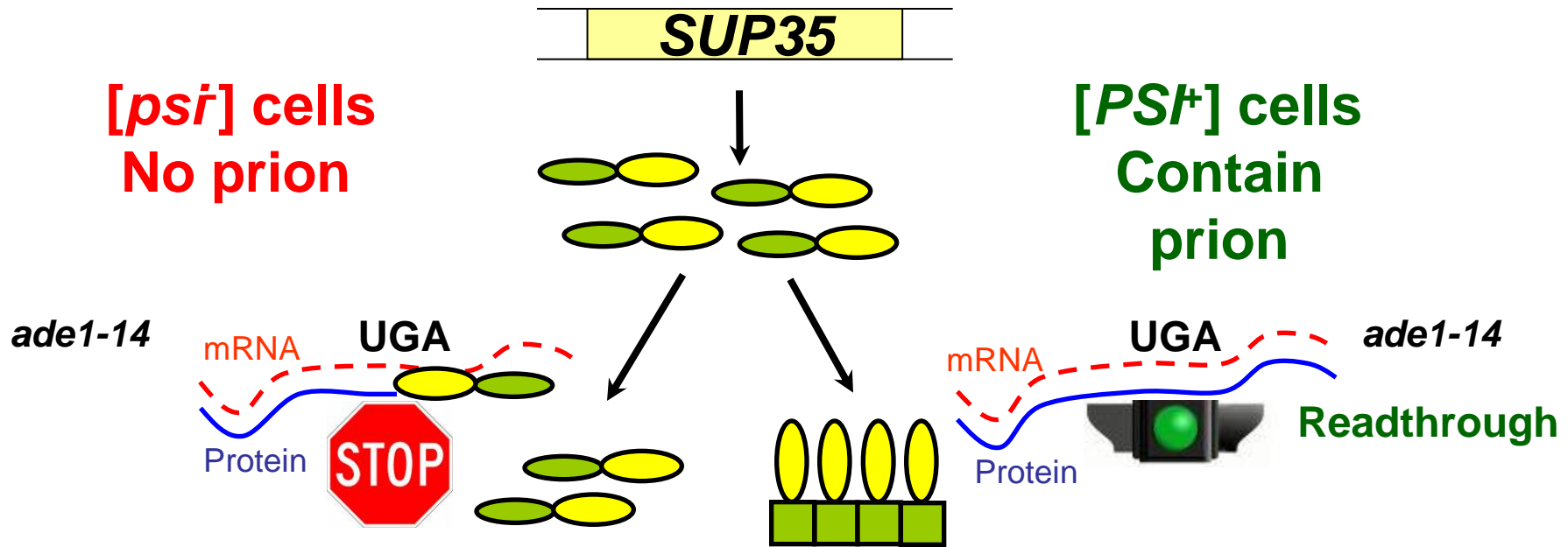
Usually at N or C terminus

YEAST PRION PROTEIN SUP35



- 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

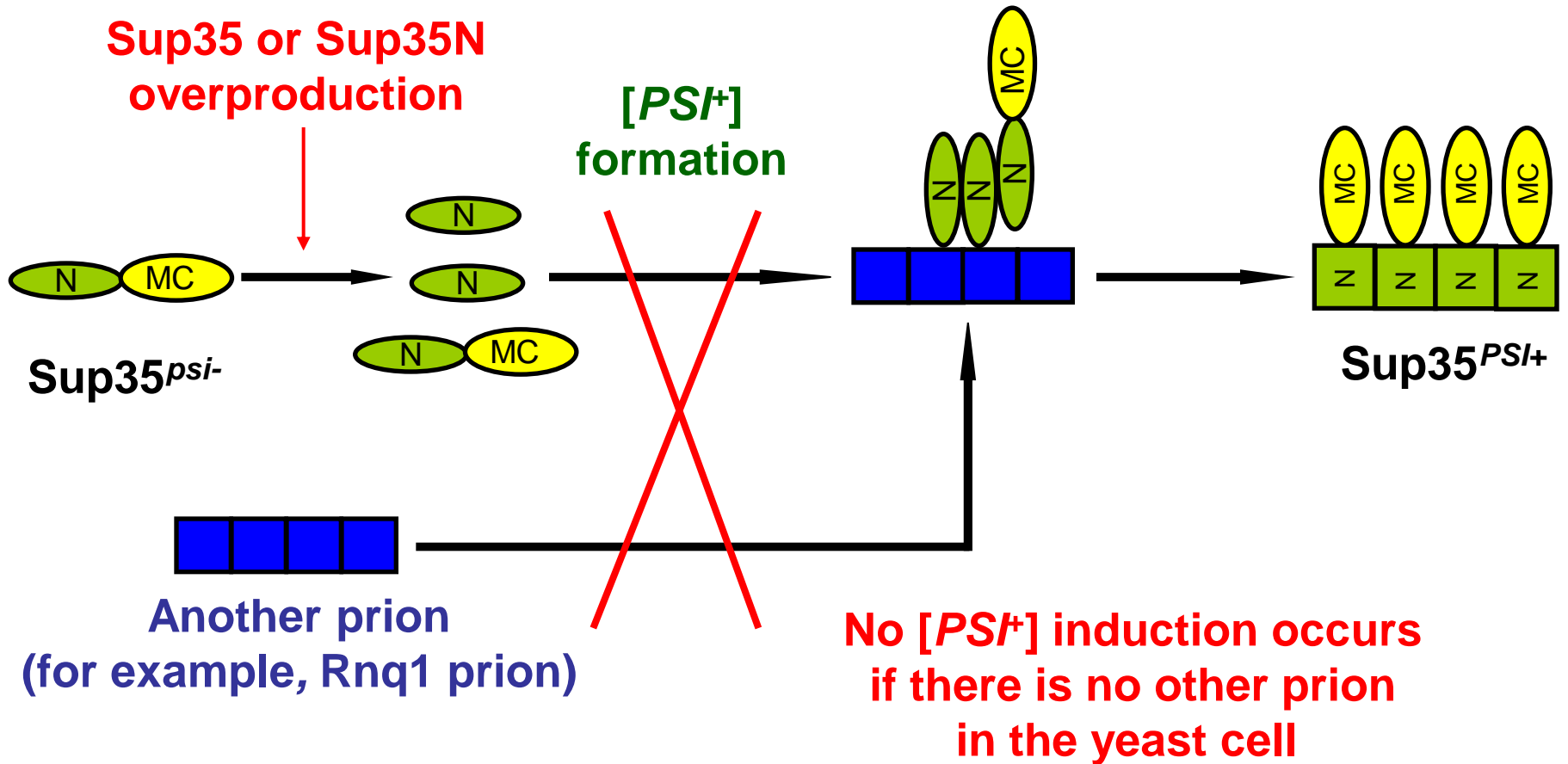
Growth on -Ade



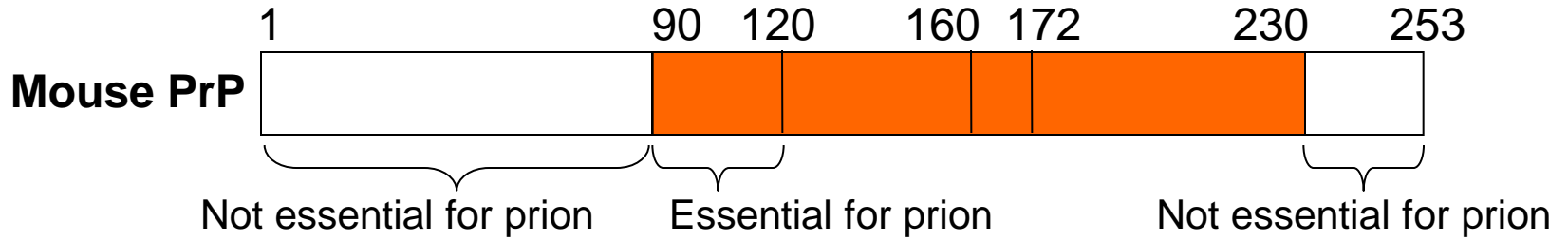
White on YPD



FORMATION OF THE [PSI⁺] PRION DEPENDS ON PROTEIN LEVELS AND ON OTHER PRIONS



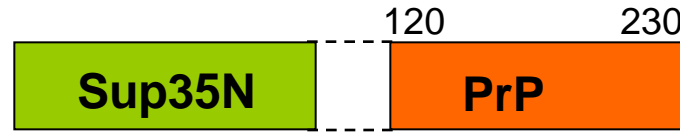
FUSION OF SUP35N TO PrP INDUCES [PSI⁺] IN THE ABSENCE OF OTHER PRIONS



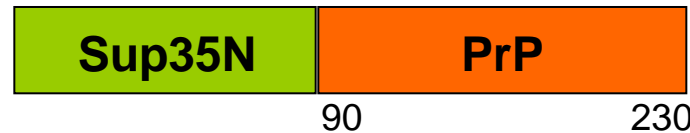
MAMMALS / HUMANS

YEAST

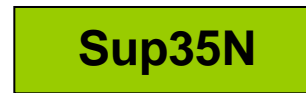
Not susceptible to prion disease



Susceptible to prion disease



Not applicable



No prion induction

Not applicable



Strong induction

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



Causes heritable prion disease



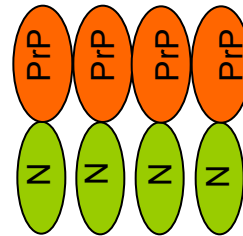
-Ade

MODEL OF PRION INDUCTION BY SUP35N-PrP IN YEAST

Prion properties of PrP drive formation of the yeast prion

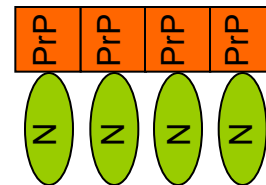
Needs 90-120 region

Oligomer formation by PrP domains



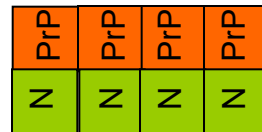
Oligomer stabilization due to conformational conversion?

Counteracted by PrP C-terminus, stabilizing the native conformation

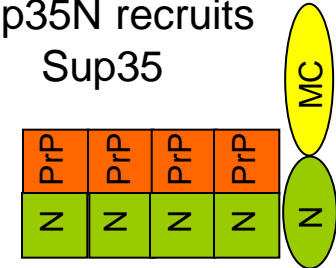


Oligomerization and conformational conversion steps are being specifically targeted

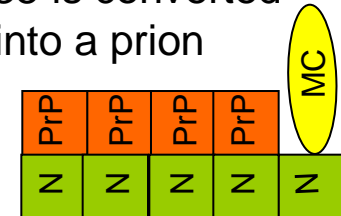
Sup35N converts into a prion state



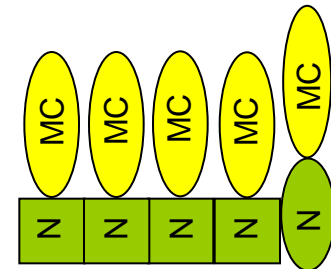
Sup35N recruits Sup35



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 promote 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**

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