Identifying Critical Sites for PrP^{C}-PrP^{Sc} Interaction Through Dominant-Negative Inhibition

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Senior Honors Project

Undergraduate Research Day

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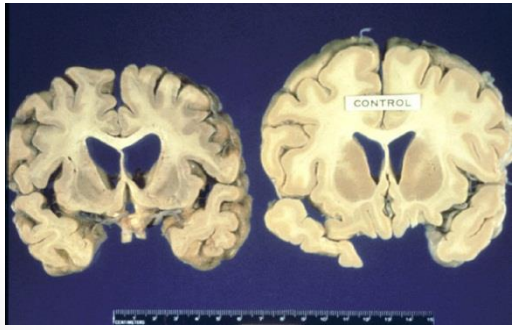
Transmissible Spongiform Encephalopathy

Prion Diseases

- Creutzfeldt-Jakob disease (Humans)
- Bovine Spongiform Encephalopathy (BSE; Cattle)
- Scrapie (Sheep)
- Chronic Wasting Disease (Cervids)

Source: Acquired, Familial & Sporadic

Universally fatal and there is no treatment



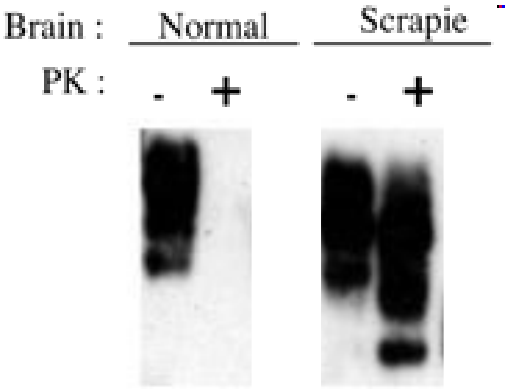
What is a prion?

Prion: Abnormally-folded Prion Protein (PrP)

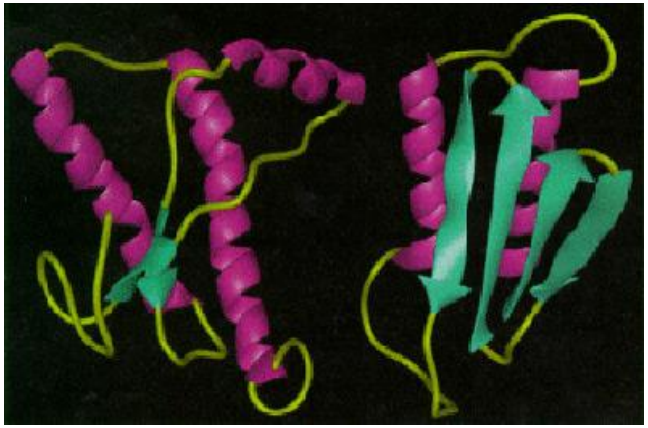
- PrP^{C}: normal cellular isoform
- PrP^{Sc}: Infectious isoform

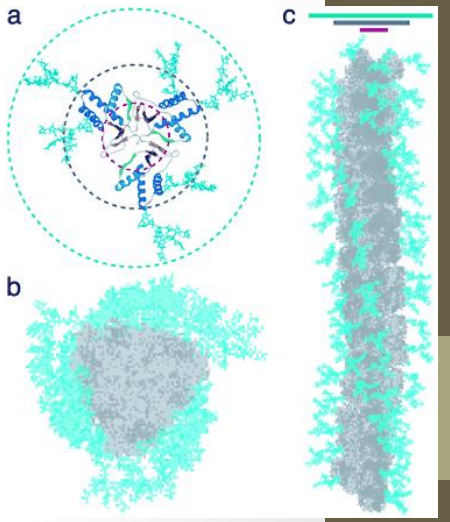
Enigma: Despite its lack of genetic material

- High Infectivity
- Various Strains



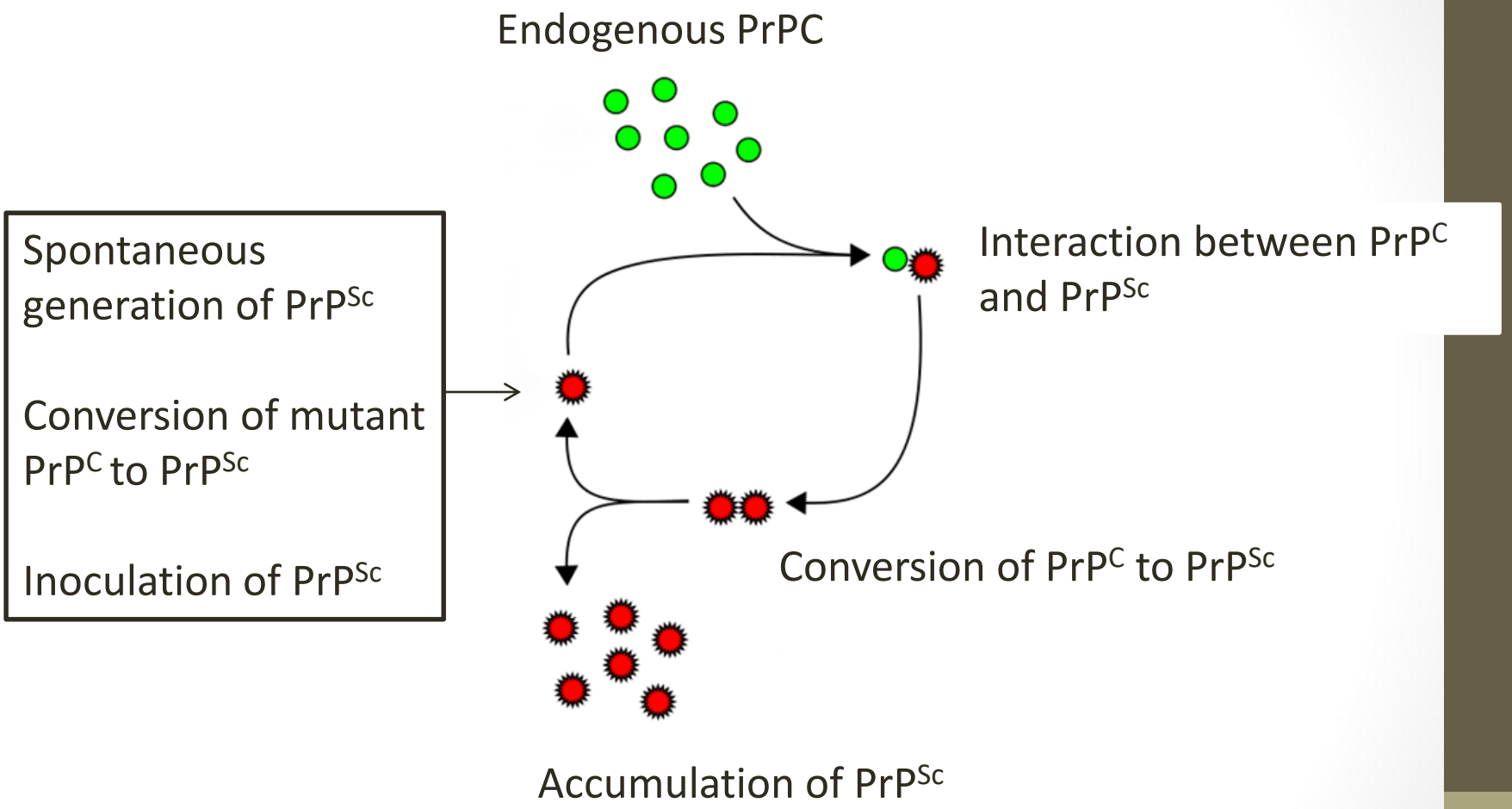
Jacquemot et al., J Virol 2005





Daggett's Model (Spiral model)

Replication of Prion (PrP^{Sc})



The conversion of PrP^{C} to PrP^{Sc} is still not completely understood.

PrP^{Sc}-PrP^{C} or PrP^{Sc}-PrP^{Sc} Interaction

- Structures of PrP^{Sc} is stable in Oligomers

  → PrP^{Sc}-PrP^{Sc} interaction

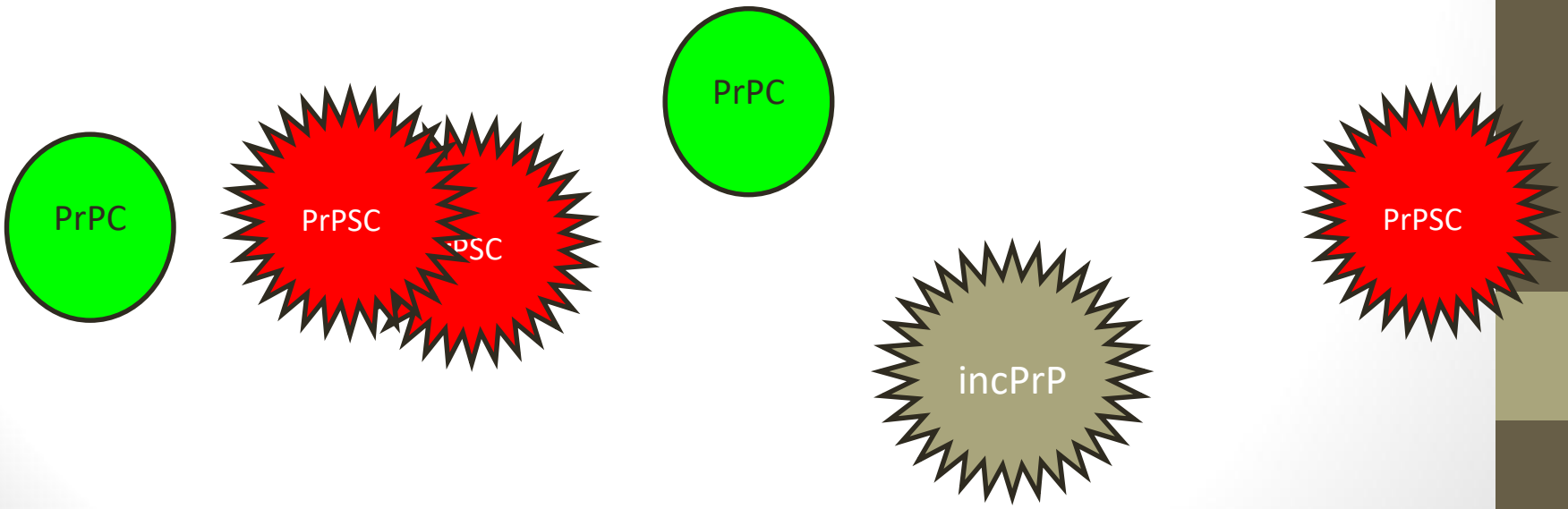
- Strain-specific structures are inherited with High fidelity

  → PrP^{C}-PrP^{Sc} interaction

Investigation into PrP-PrP interactions are important

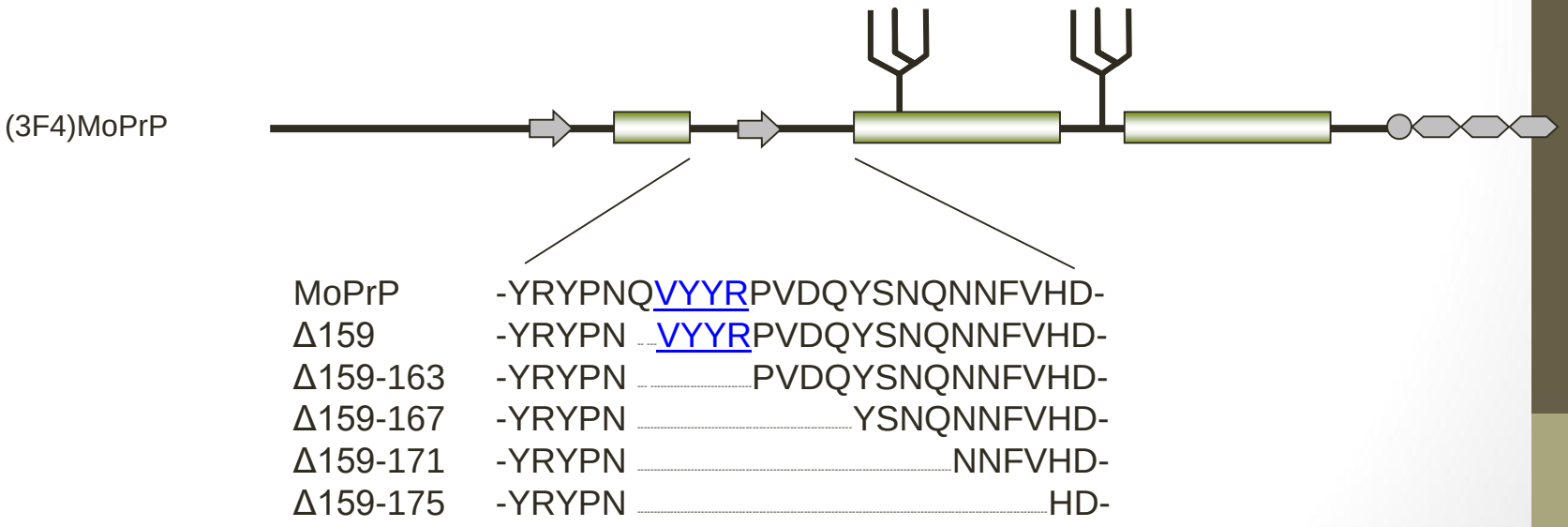
Dominant-Negative Inhibition

- When a conversion-incompetent PrP (incPrP) co-exists with a conversion-competent PrP, the former inhibit the conversion of the latter.
- Efficiencies of the inhibition varies between incPrPs
- Due to variation in Binding affinity of incPrPs to PrPSc?



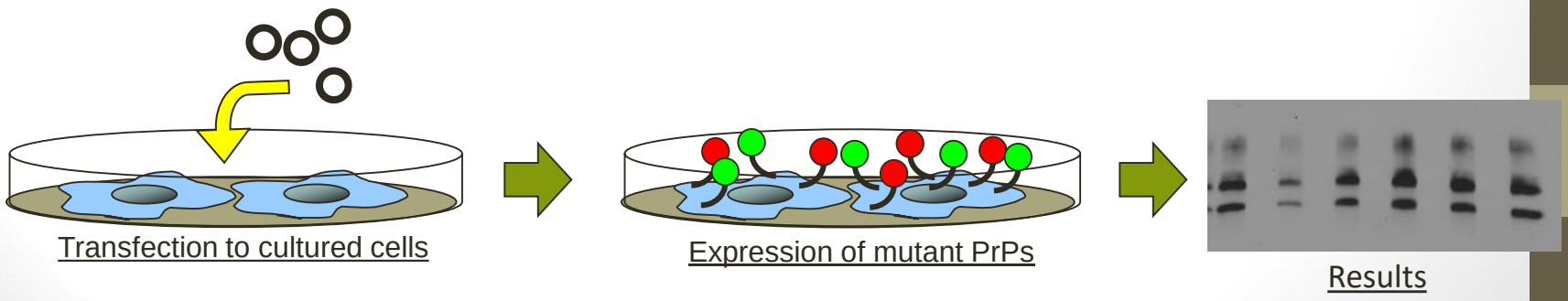
Hypothesis

If efficiency of dominant-negative inhibition depends on binding affinity of inPrP to PrP^{Sc}, the region necessary for PrP^{C}-PrP^{Sc} interaction can be identified by making a series of Internal-deletion mutants and evaluating their inhibitory effects.



Methods

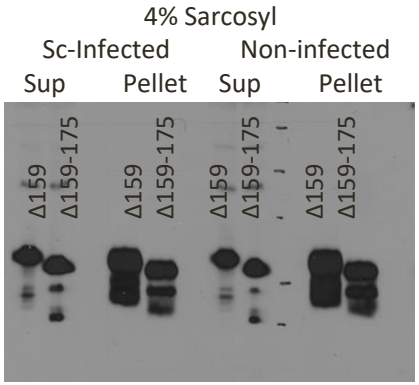
- Create Internal-deletion PrPs (ΔPrPs) by site-directed mutagenesis
- Amplify the plasmids encoding ΔPrPs in competent cells and purify the plasmids
- Transfect the plasmids to scrapie-infected cultured cells (Co-express conversion-competent PrP with ΔPrP).
- Harvest, SDS-PAGE & immunoblot to evaluate dominant-negative inhibition



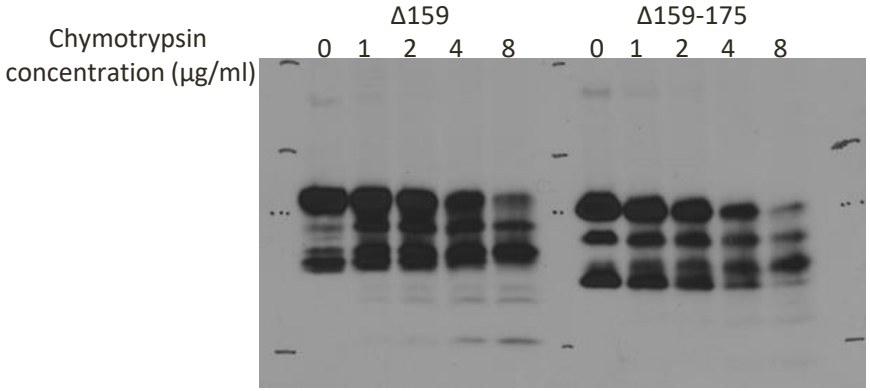
Results

- ΔPrPs showed insolubility to detergent & mildly protease-resistant unlike wild-type PrP

4H11 (Endogenous Wild-type PrP)



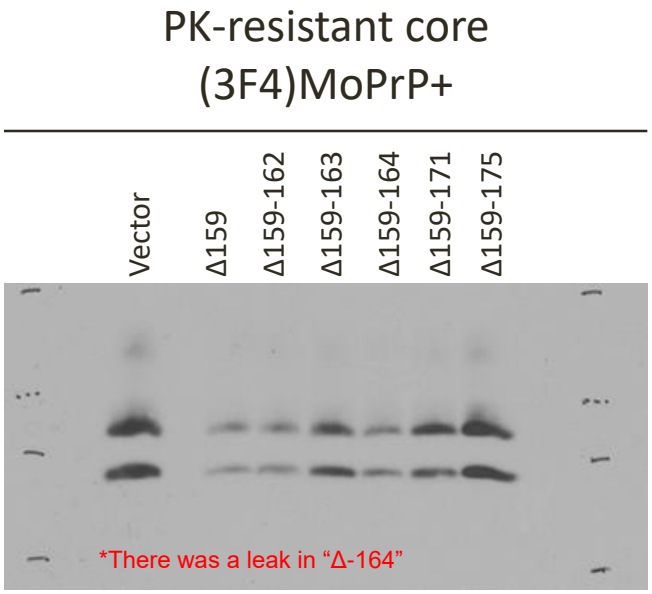
3F4 (Transfected ΔPrPs)

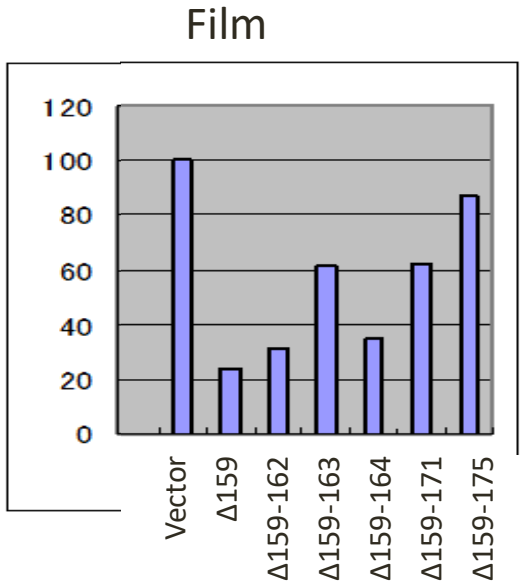


These findings suggests that ΔPrPs might be different from wild-type PrP but there was no difference among ΔPrPs.

Results

Co-transfected (3F4)MoPrP with the Internal-deletion mutants and observed the levels of (3F4)PrP^{Sc}

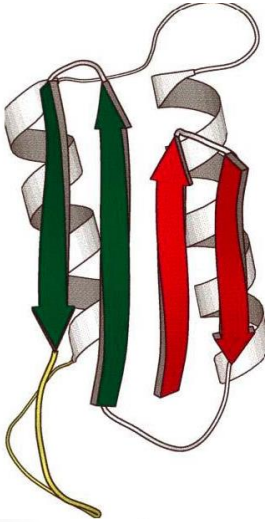


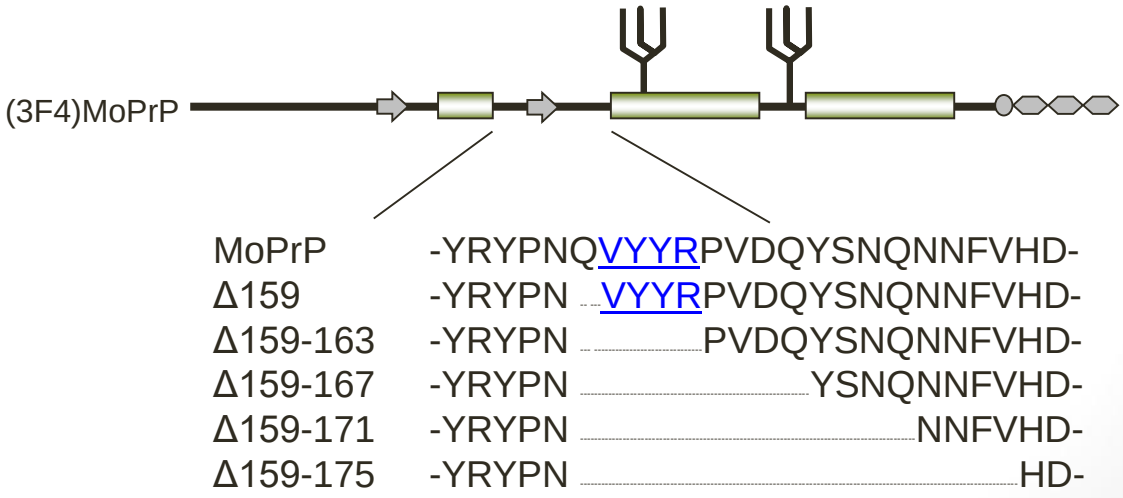


Δ159 and Δ159-162 showed most efficient inhibition and the inhibitory effects were reduced as the deletions were extended

Discussion

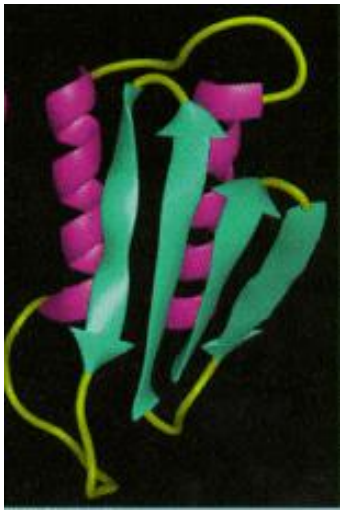
- Reproducible differences between ΔPrPs in efficiencies of dominant-negative inhibition were observed
- The differences suggests that the loop region between the second beta strand and the second alpha helix might be important for efficient inhibition
- This supports the point of view that the loop region is important for propagation of prion/PrPSc

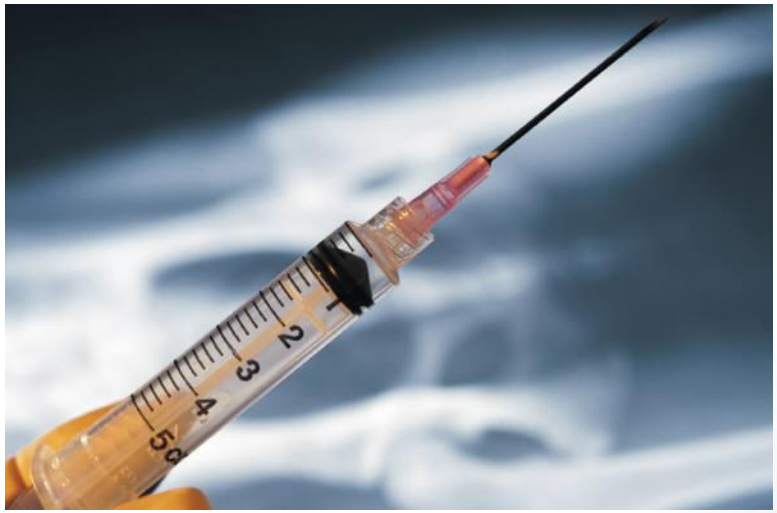




To be continued….

- Isolation of the critical amino acids that are absolutely needed for binding (proteins following the B-sheet)
- Finding efficient incPrPs that can bind to PrP^{Sc} effectively may be used therapeutically by the inhibition of PrP^{C} to PrP^{Sc} conversion.





Acknowledgments

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