International Conference on Emerging Infectious Diseases 2002

The Agent: Prions as Emerging Infectious Particles

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Topics Covered

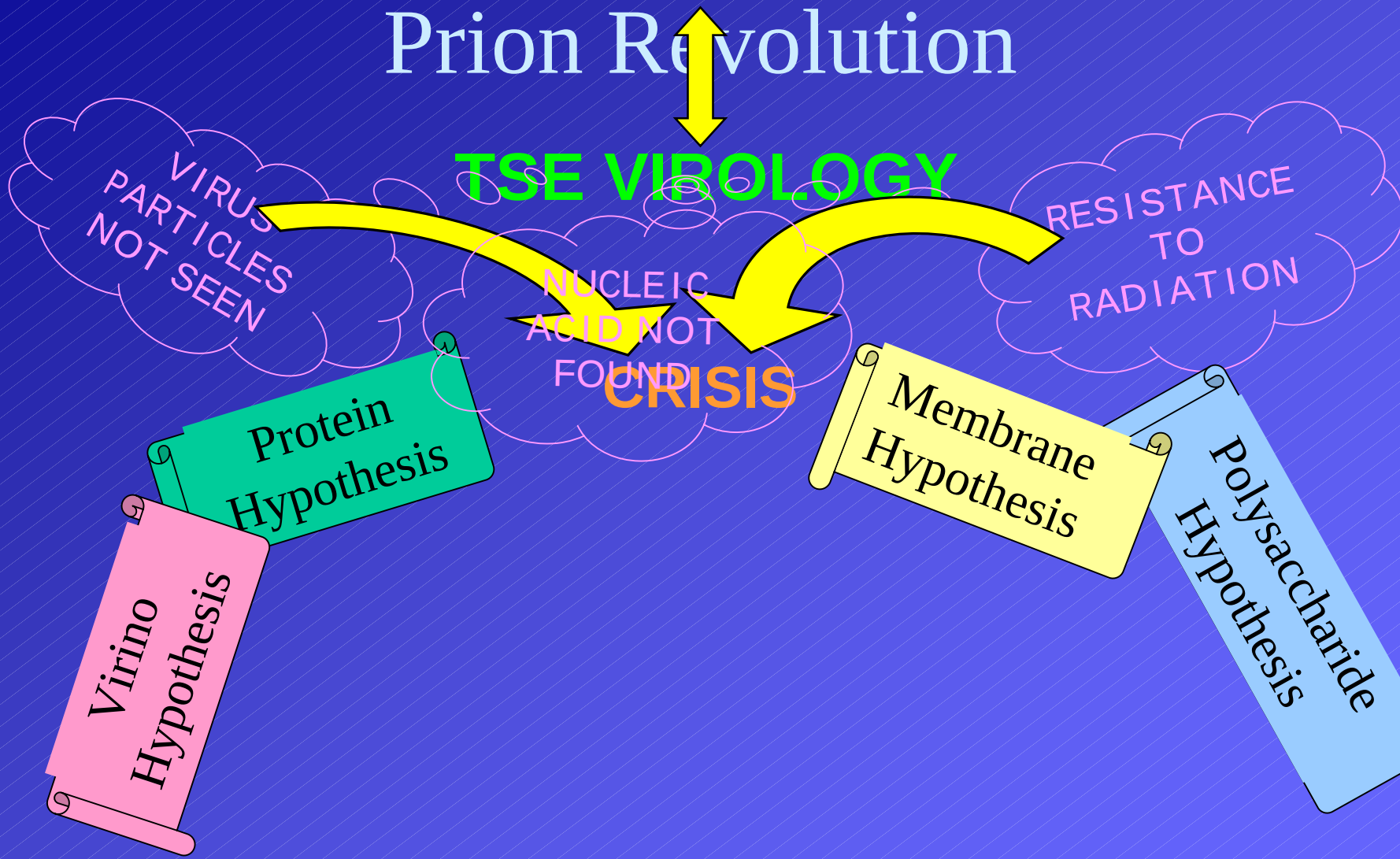
- Prions
  - Prion paradigm shift
  - Prion composition and structure
  - Prion replication
- Prions as emerging infectious particles
  - Prion diseases
  - Emerging prion diseases

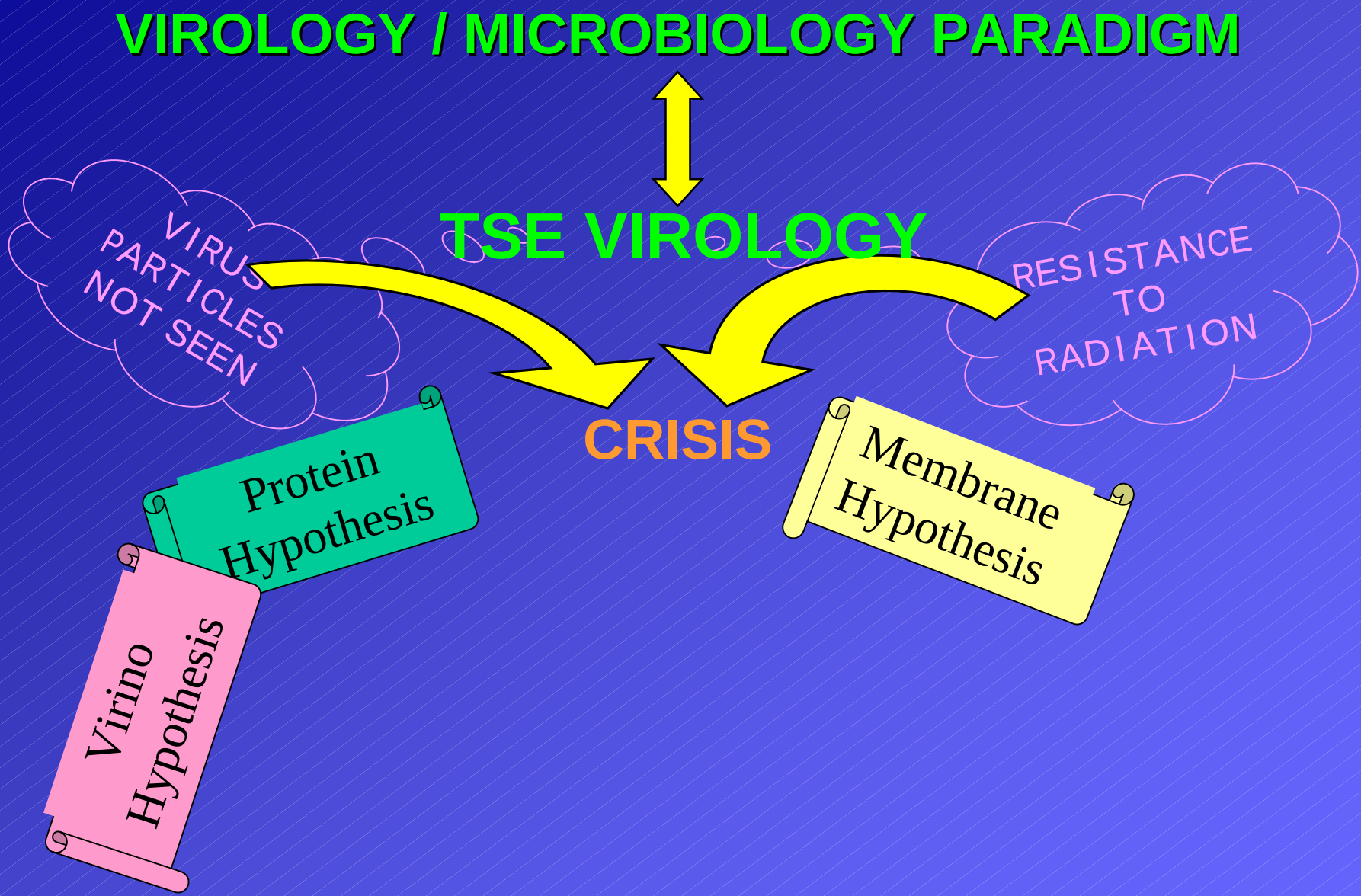
Kuhn's Scientific Revolutions

- Paradigm-based normal science
- Crisis provoked by anomalous observations
- Revolution
  - Competition between new and old paradigms
  - New paradigm displaces old
- Normal science under a new paradigm
  - Transition is discontinuous
  - New or revised field is repopulated with believers

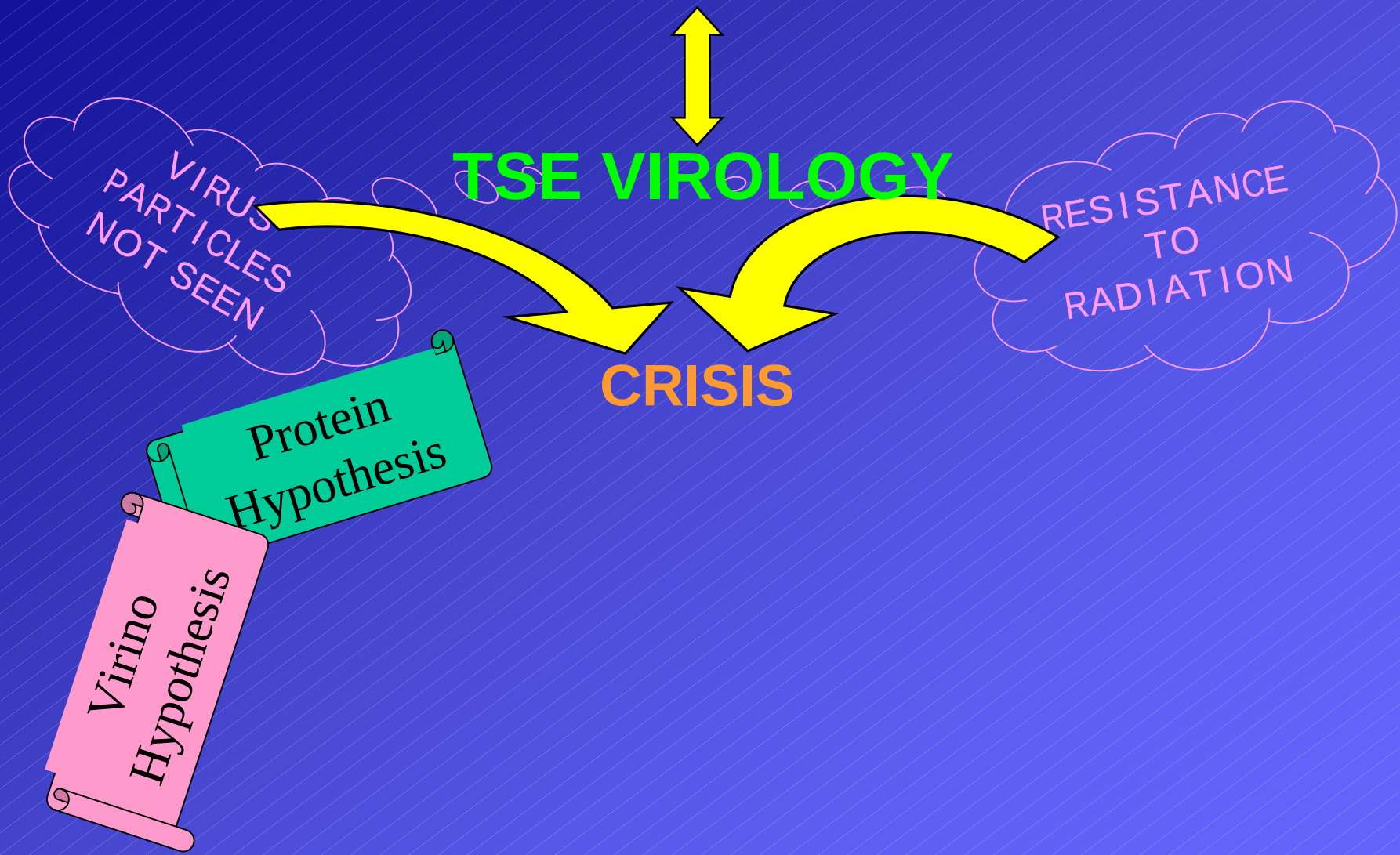
VIROLOGY / MICROBIOLOGY PARADIGM

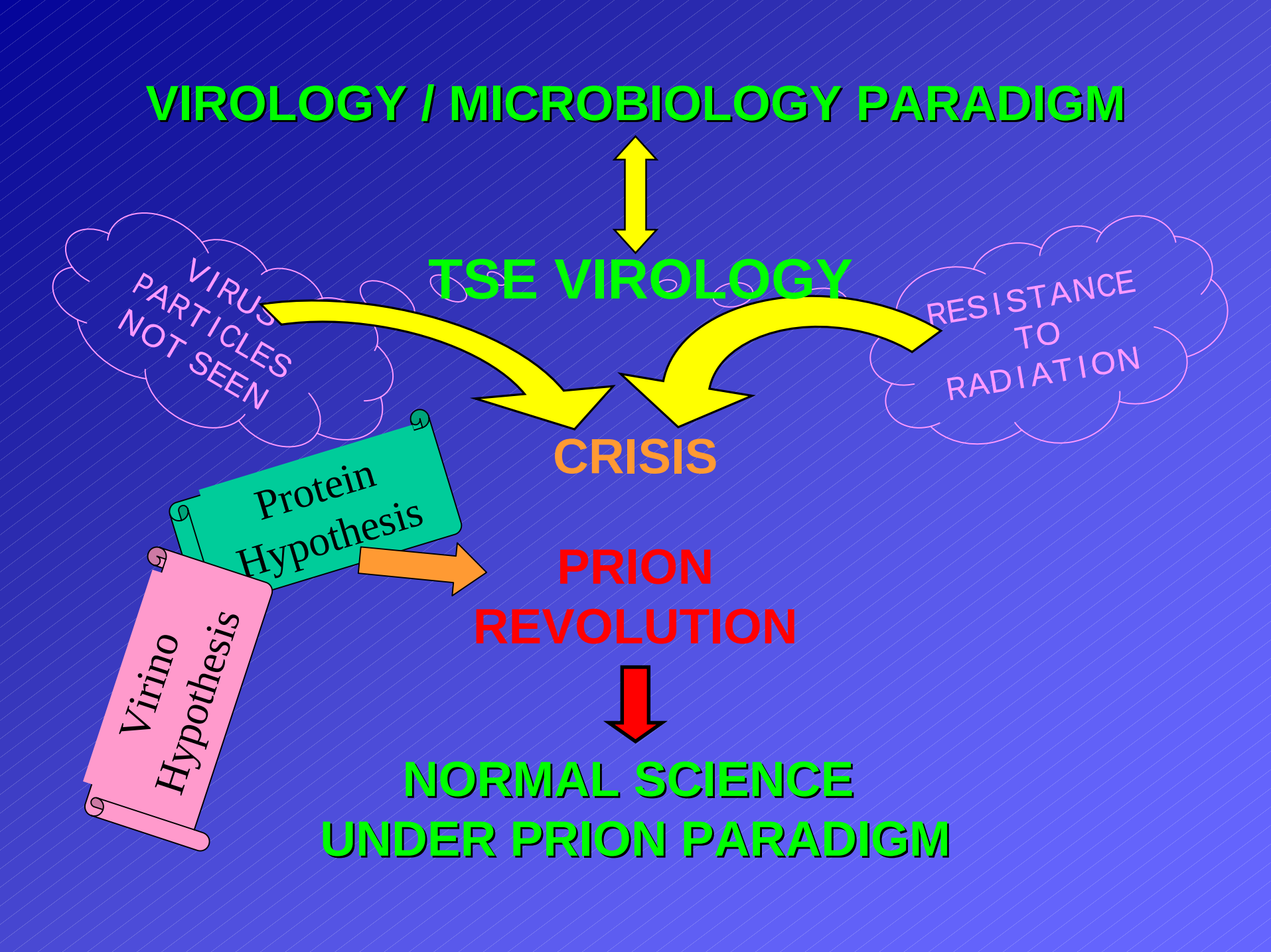
Prion Revolution




VIROLOGY / MICROBIOLOGY PARADIGM
TSE VIROLOGY
VIRUS PARTICLES NOT SEEN
RESISTANCE TO RADIATION
CRISIS
Protein Hypothesis
Membrane Hypothesis
Virino Hypothesis

VIROLOGY / MICROBIOLOGY PARADIGM

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PRION REVOLUTION
NORMAL SCIENCE UNDER PRION PARADIGM

Prion Paradigm Shift

- Prion paradigm displaced virus paradigm
  - Prion paradigm is better at explaining the existing data
  - Prion paradigm is not necessarily correct
- Shift to prion paradigm takes time
  - Young scientists or those from other fields tend to adopt the prion paradigm
  - Established scientists tend to resist the prion paradigm
  - Crisis began ~1965; gained wide acceptance ~1990's
  - Transition to new paradigm depends on the individual

Prion (Protein) Hypothesis

- Prions are infectious agents composed of a modified host protein, PrP^{Sc}
- A non-host nucleic acid is not a component
- PrP^{Sc} is derived from the cellular form, PrP^{C}
- PrP^{Sc} and PrP^{C} share the same primary structure
- PrP^{Sc} and PrP^{C} have different conformations

Unusual Properties of Prions

Early Studies

- Resistant to UV and ionizing radiations
- Resistant to various chemical and enzymatic treatments
- Heterogeneous in size and density
- No unitary structure identified by EM

Unusual Properties of Prions
Later Studies

- Scrapie-associated fibrils (SAF) identified by EM
- Abnormal host protein (PrP^{Sc}) purifies with infectivity
- Degrading PrP^{Sc} destroys infectivity
- Denaturing PrP^{Sc} destroys infectivity
- Purified preps lack specific nucleic acid

PrP^{Sc} and Prions

- PrP^{Sc} and infectious prions copurify
- PrP^{Sc} is the only macromolecule consistently identified in purified prion preparations
- No prion-specific nucleic acid has been identified (except host PrP gene & mRNA)
- Prions are unaffected by many treatments that degrade nucleic acids

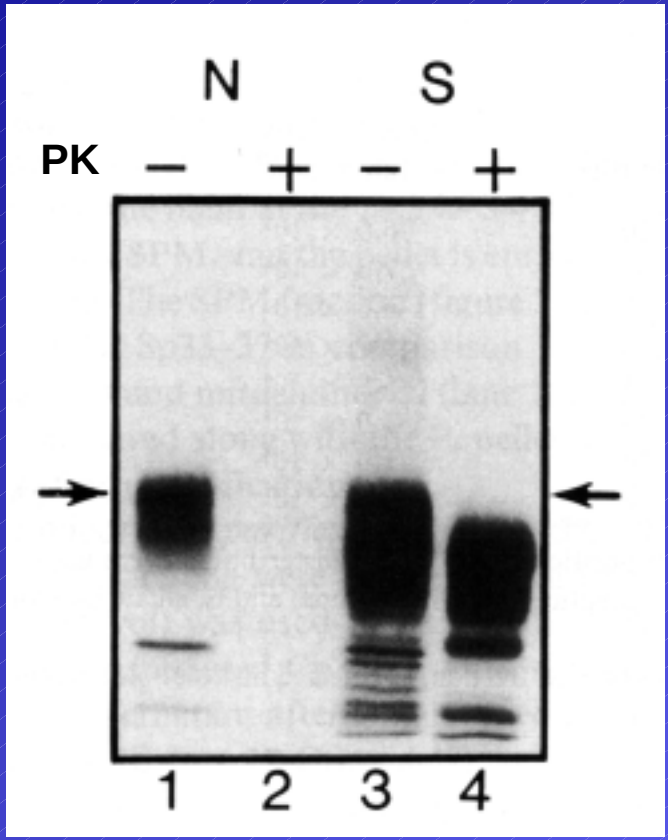
PrP^{Sc} and Prions (2)

- PrP^{Sc} and prions resist degradation by proteases
- Prolonged digestion degrades PrP^{Sc} and destroys infectivity
  - Kinetics of degradation of PrP correlate with prion inactivation
- Denaturing PrP^{Sc} destroys infectivity
  - Physical treatments
  - Chemical treatments

PrP^{Sc} and Prions (3)

- PrP^{Sc} concentration and prion infectivity correlate in various preparations
- PrP^{Sc} binds to PrP^{C} *in vitro* and can change its conformation
- Mice that don't have the PrP gene (*Prnp*$^{0/0}$) cannot be infected by prions
- Neurons that do not produce PrP^{C} are not damaged by prion infection

Properties of PrP^{C} and PrP^{Sc}

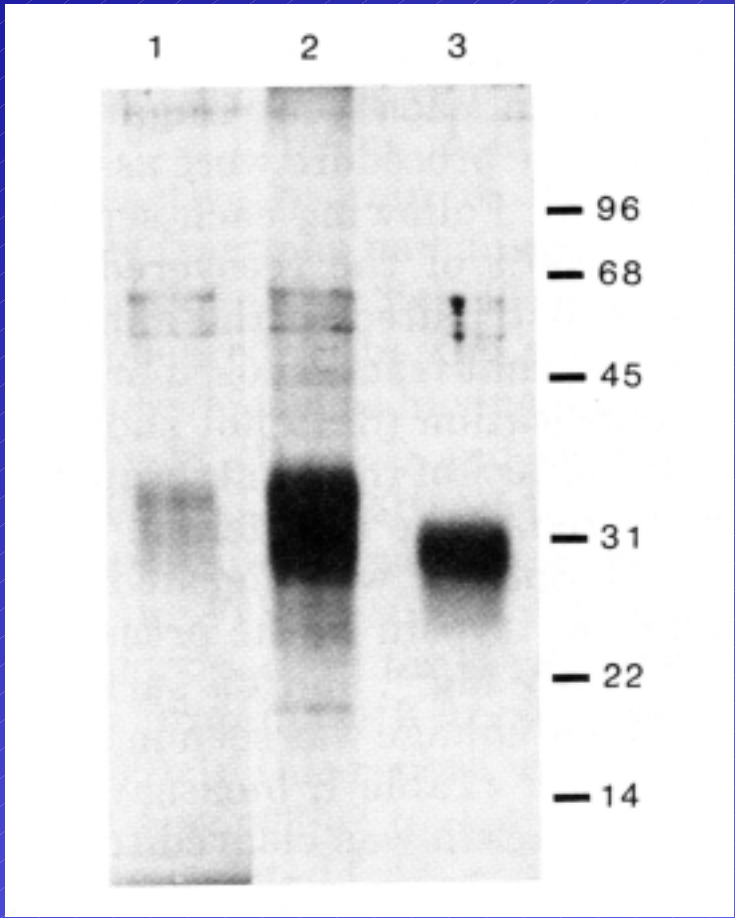


PrP detected by Western blotting

Characteristic	PrP^{C}	PrP^{Sc}
Normal brain	Present	Absent
Diseased brain	Present	Present
Infectious	No	Yes
Concentration (μg/g)	20 – 40	60 – 160
Fibrils in vivo	No	Yes
Amyloid plaques	No	Yes
Soluble in Sarkosyl	Yes	No
Resists proteolysis	No	Yes

Bendheim *et al*. (1988) J.Infect.Dis. 158: 1198-1208

Purified PrP^{Sc} is Infectious

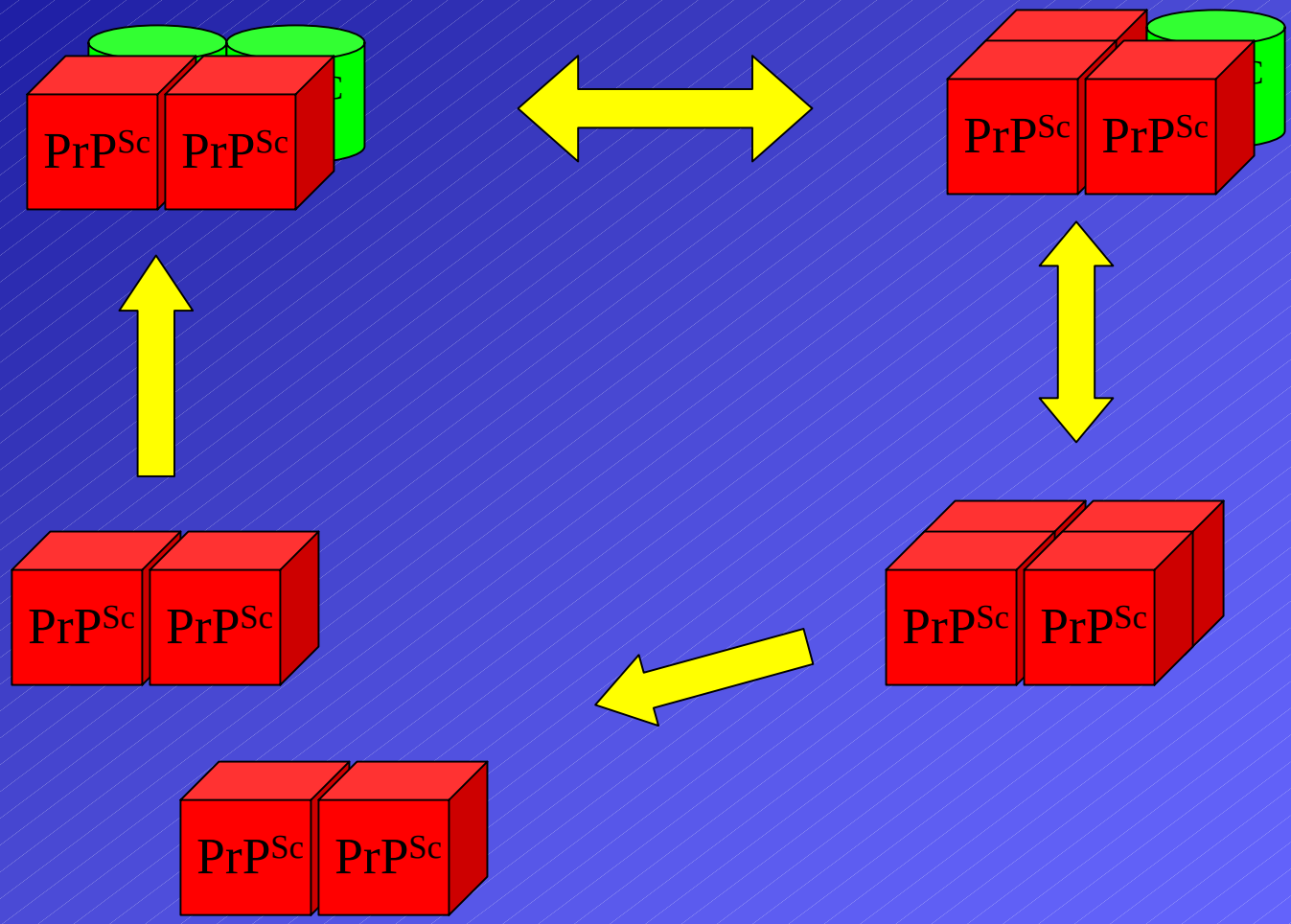


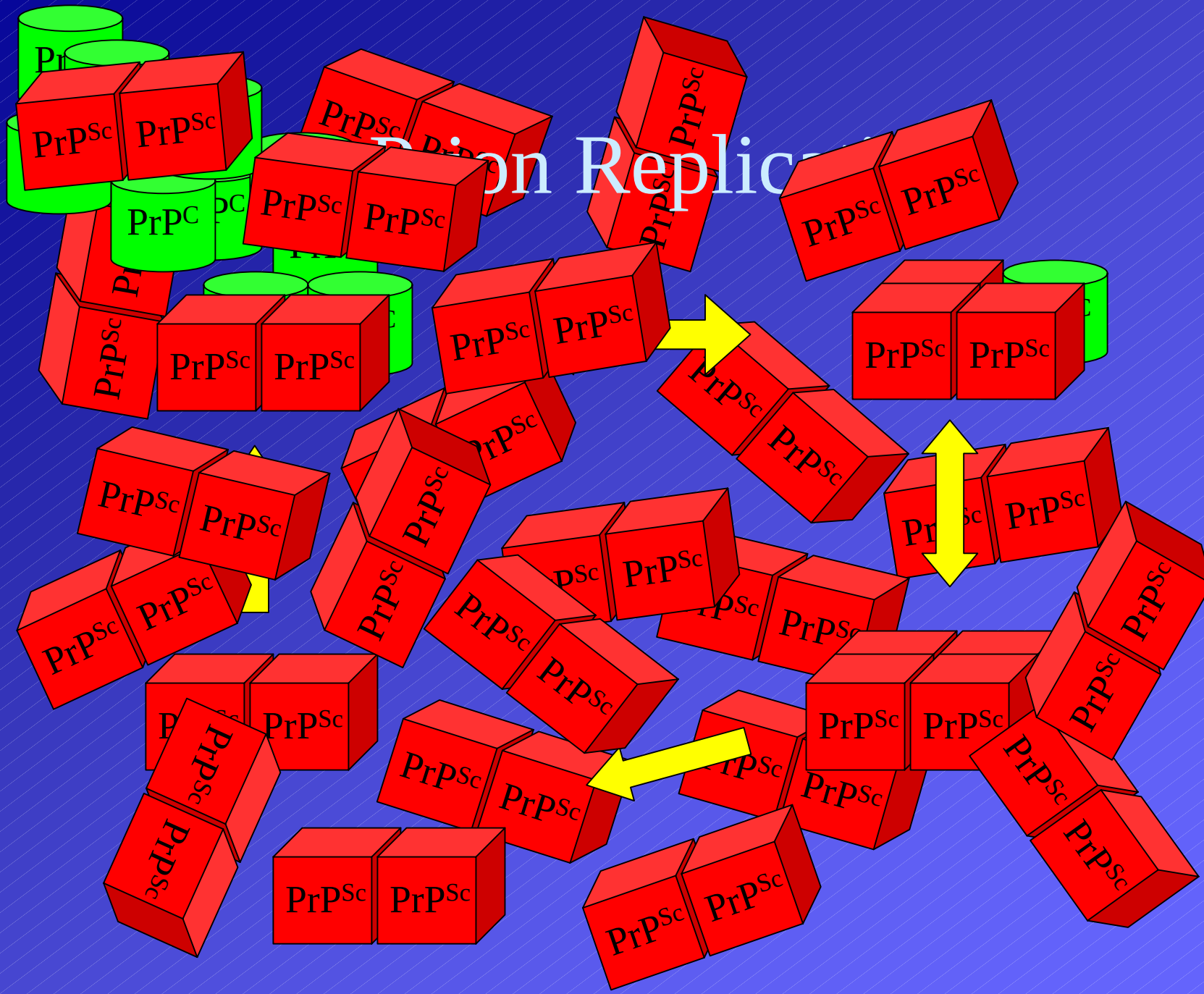
*PrP detected by silver staining

Sample	Specific Activity (LD_{50}/mg protein)
PrP^{Sc}	
Lanes 1 & 2* (Lane 2 contains 4 times as much PrP as lane 1)	6×10^{10}
Trypsin fragment of PrP^{Sc}	
Lane 3*	3×10^{11}

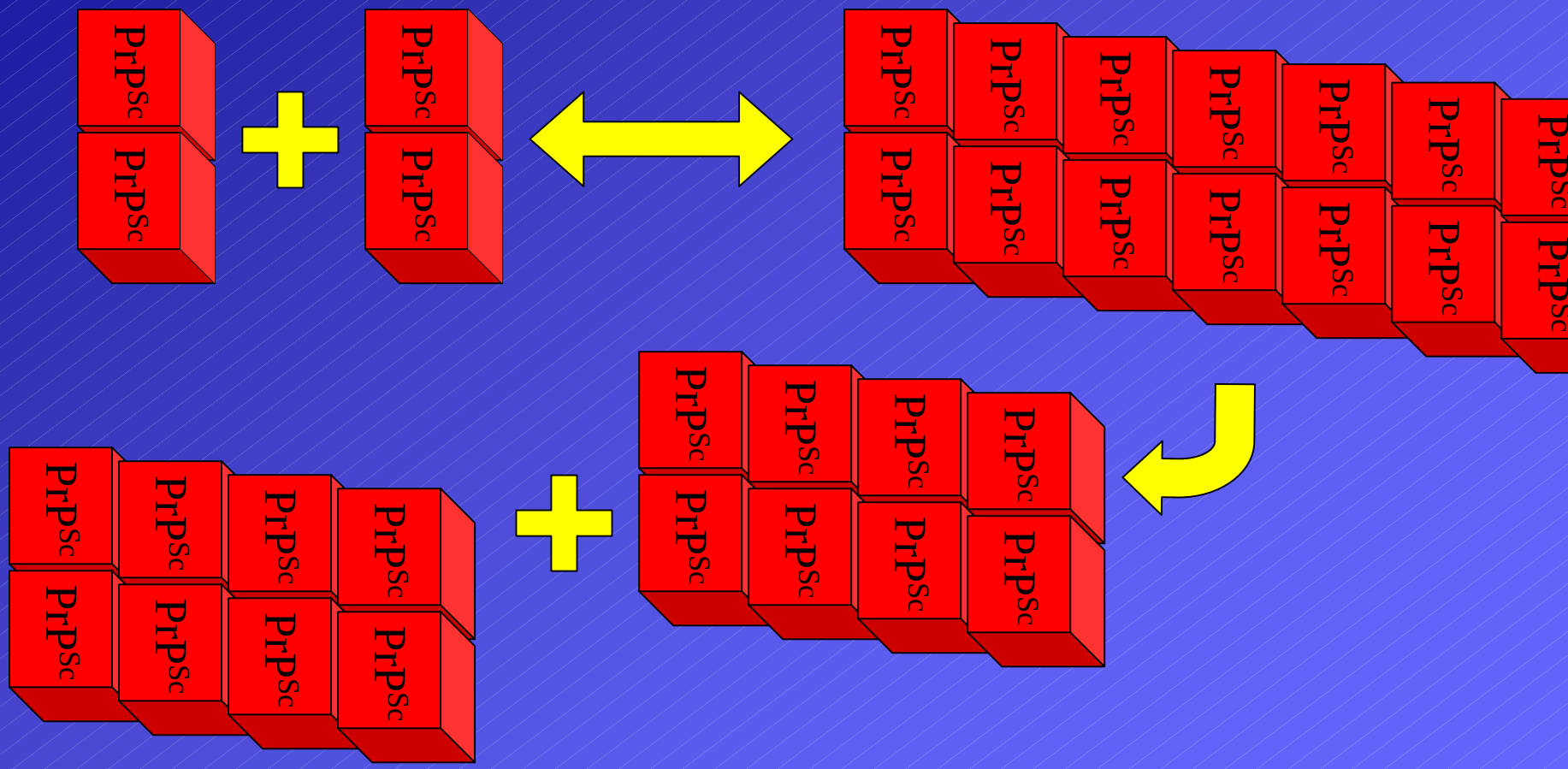
Bolton, *et al*. (1987) Arch. Biochem. Biophys. 258: 579-590

Prion Replication




Prion Replic
PrPSc
PrPC

Prion Aggregation Dynamics



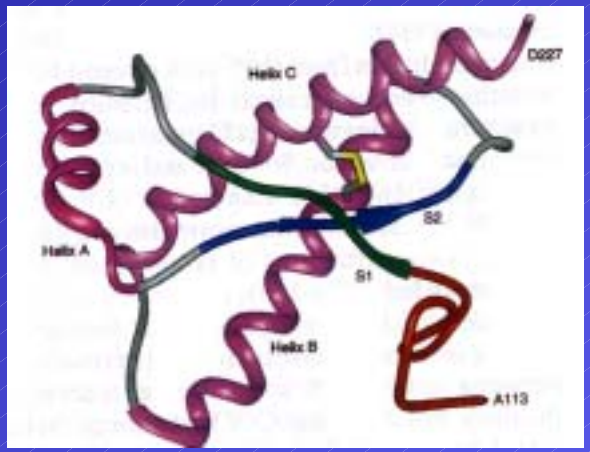
Prion Structural Models

PrP^{Sc} binding & conversion

PK + detergent

PrP^{C} ⇌ PrP^{Sc} → PrP 27-30

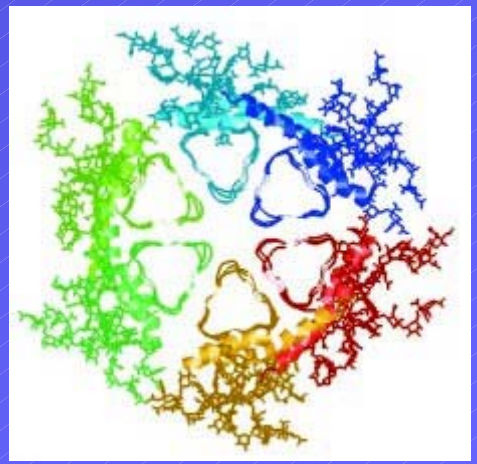
NMR Structure



Prusiner, et al. (1998) PNAS 95: 13363-13383

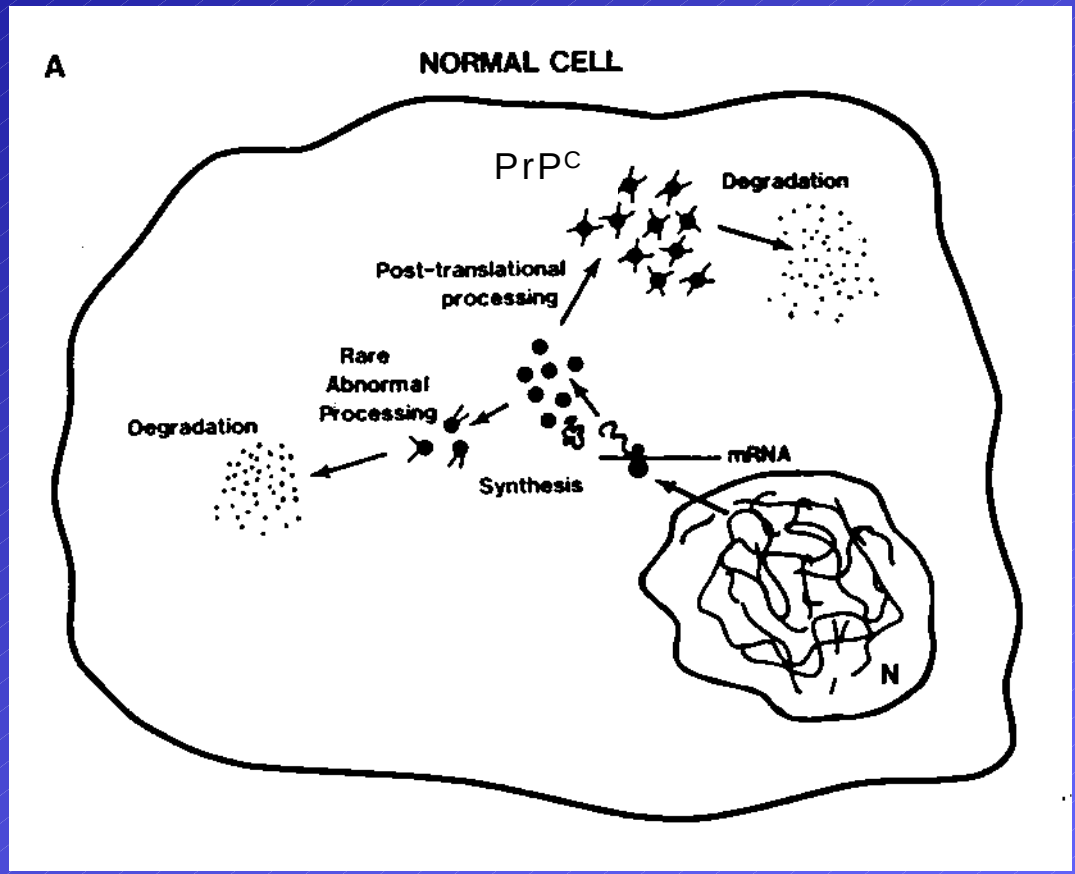
?

Electron Crystallography Structural Model



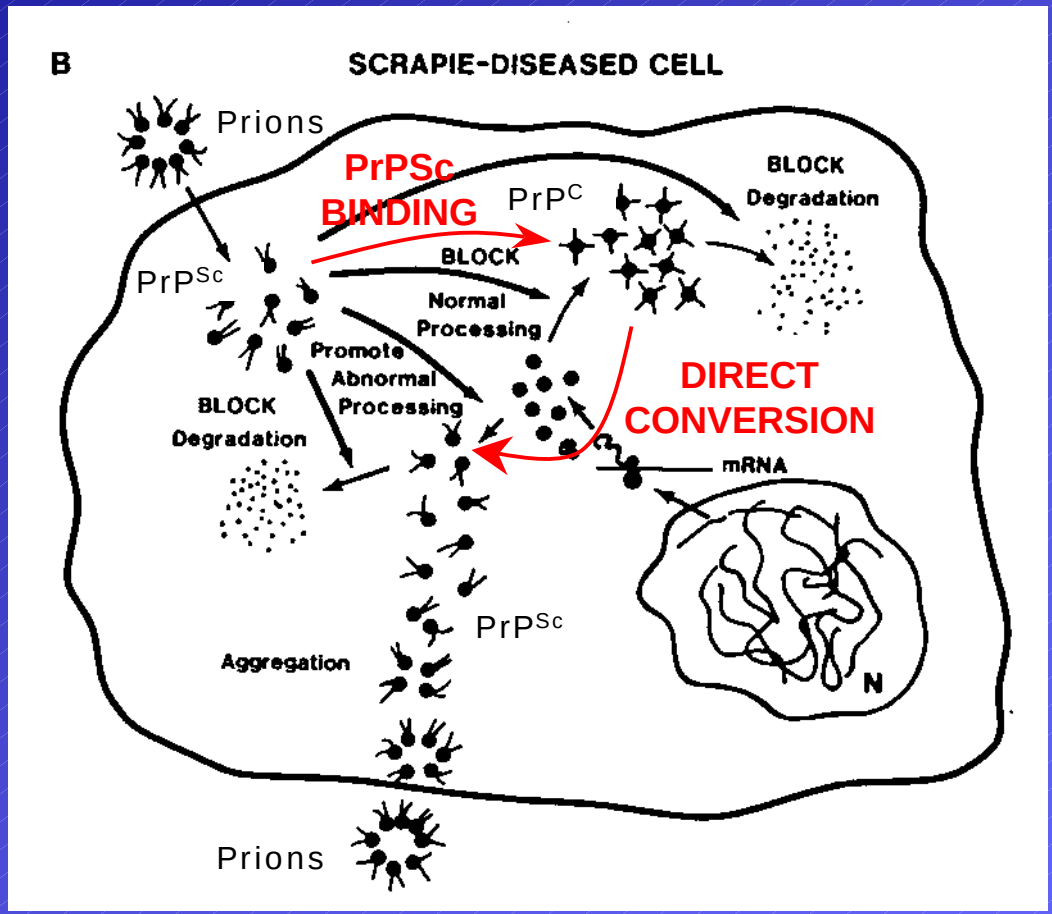
Wille, et al. (2002) PNAS 99: 3563-3568

PrP^C Metabolism in a Normal Cell



Bolton & Bendheim (1988) Ciba Found. Symp. 135: 164-181

Possible Sites of PrP^{Sc} Action



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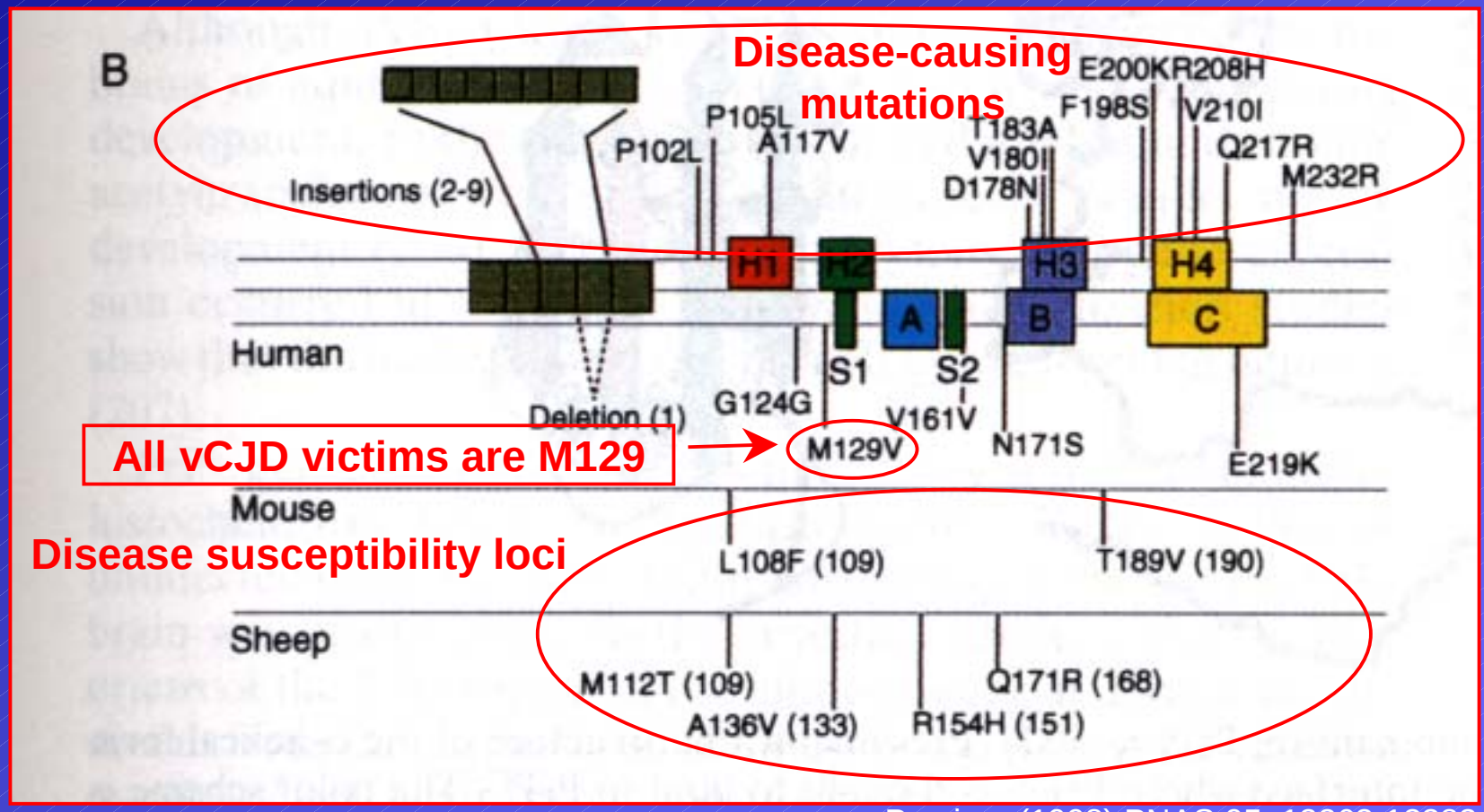
Summary

- Prions are composed of an abnormal host protein, PrP^{Sc}
- Conformation of PrP^{Sc} is different from host PrP^{C}
- Prion-specific nucleic acid molecules have not been identified
- Protein-only hypothesis provides the best explanation for data (to date)

Prion Disease Etiologies

- Familial
  - More than 25 mutations in *PRNP* gene
  - Autosomal dominant mutations
  - Some cases are infectious (transmitted experimentally)
- Infectious
  - Transmissible vs. contagious
  - Biologically distinct strains
  - Infectious particle contains PrP^{Sc} from wild-type gene
- Sporadic
  - No demonstrable link to familial or infectious causes

Species Variation and Mutations



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Infectious Prion Diseases

Animal Diseases

- Scrapie
- BSE
- CWD
- TME
- FSE
- Exotic ungulate encephalopathy

Human Diseases

- Variant CJD (vCJD)
- Iatrogenic CJD (iCJD)
- kuru
- sCJD as source for
  - iCJD
  - kuru)
  - ???

Emerging Prion Diseases

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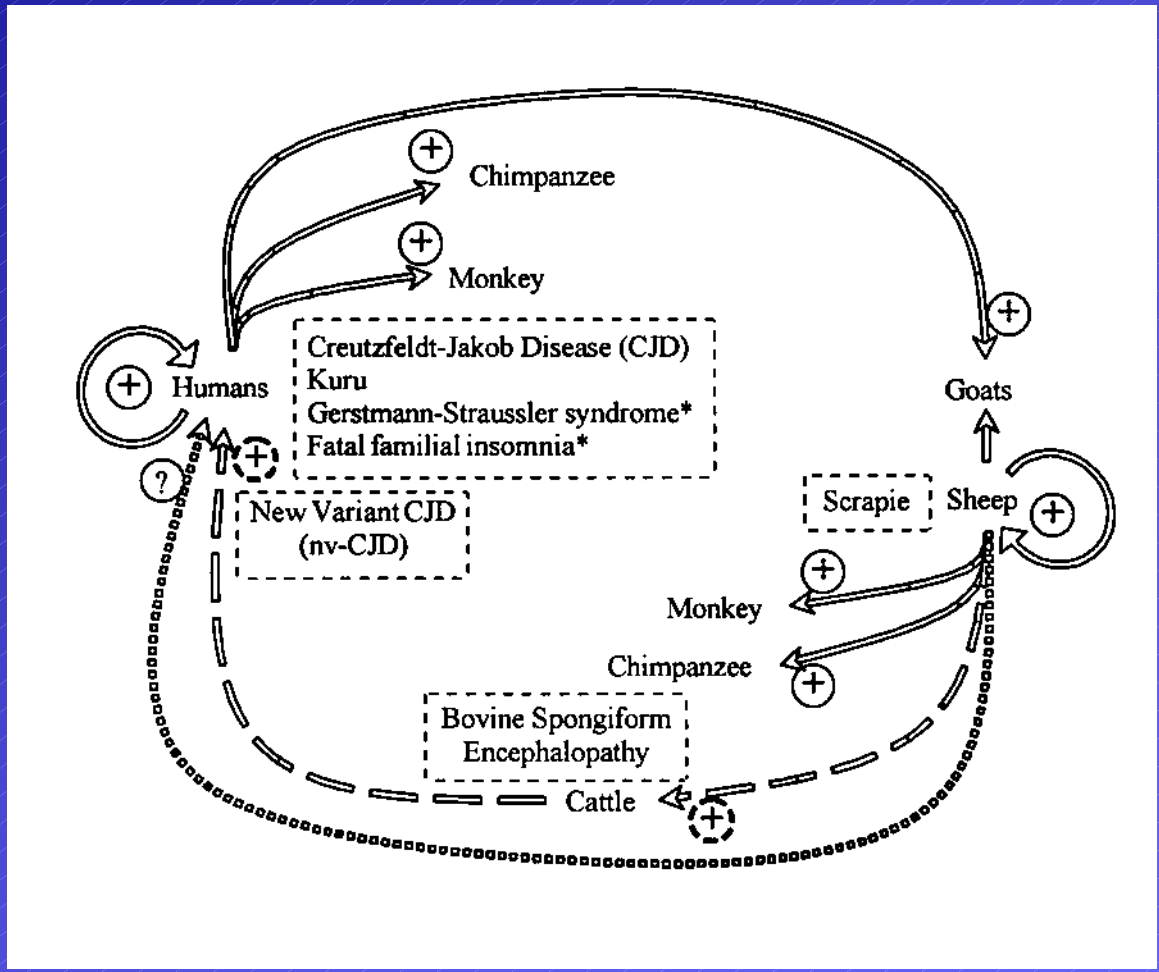
Interspecies Transmission of Prions



Prions as Agents of Emerging Disease

Good News

- Long incubation times
  - Time to react
- Not highly contagious
- Prions show restricted tissue distribution
- Species barriers

Bad News

- Long incubation times
  - Undetected reservoirs
- Invariably fatal
- Difficult to inactivate
- Persist in environment
- Difficult to detect
- High prion titers in CNS

Revolution

"I have shown the existence of at least three classes of replication mechanisms and that, therefore, the occurrence of a protein agent would not necessarily be embarrassing although it would be most interesting."

J.S. Griffith *Nature 215: 1043-1044 (1967)*

Revolution (2)

"This shows that there is no reason to fear that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down."

J.S. Griffith *Nature 215: 1043-1044 (1967)*