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PRION PROTEIN AS AN INFECTIOUS AGENT OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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ABSTRACT

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of human, animal and yeast caused by an infectious agent designated prion. The "protein only" hypothesis proposed more than three decades ago suggests that the prions are small, infectious pathogens composed of aggregated, abnormal forms of a protein encoded in the host genome. PrP^C plays primary role in neurite outgrowth, synaptic function, oxidative stress defense and long term survival of cerebellar neurons. The key event in prion disease is the conversion of the α -helical, cellular isoform of the prion protein to the insoluble, β -sheet-rich disease-causing isoform. Aggregation of misfolded prion protein into large amyloid plaques and fibrous structures is associated with neurodegeneration. TSEs attack the central nervous system leading to progressive spongiform degeneration of brain tissue. Molecular and cellular pathways leading to neurodegeneration remains still unclear. However, the recent studies suggest that pathway leading to neuronal loss include ubiquitin-proteasome and endosomal system, oxidative stress, regulated activation of complement, synaptic alterations and dendritic atrophy. The study of the unusual properties of the infectious agent and pathology of neurodegenerative disorders has been a source of excitement and arguments between

the scientists. The major advances in molecular genetics and recent understanding of prion propagation and neurotoxicity allowed deep insight into prion biology.

TABLE OF CONTENTS

ABSTRACT.....	I
LIST OF FIGURES.....	III
CHAPTER	
I. INTRODUCTION	1
II. PRIONS	3
2.1. Discovery	3
III. CELL BIOLOGY OF PrP ^C	6
3.1. PrP ^C	6
3.1.1. Processing.....	6
3.1.2. Structure	8
3.1.3. Function	10
3.1.4. Cellular trafficking.....	10
3.2. PrP ^{Sc}	12
IV. CONVERSION OF PrP ^C INTO PrP ^{Sc}	14
V. PRION STRAINS	17
VI. SPECIES BARRIER.....	20
VII. PRIONS DISEASES	20
7.1. Spreading of disease	22

7.2. Type of prion diseases.....	24
7.3. Protein aggregation and disease.....	25
7.4. Mechanism of cell death.....	26
7.5. HUMAN PRION DISEASES.....	28
7.5.1. Creutzfeldt-Jacob disease (CJD).....	28
7.5.2. Kuru.....	30
7.5.3. Fatal familial insomnia (FFI).....	31
7.5.4. Gerstman-Straussler-Scheinker disease (GSS)	32
7.5.5. Alpers' disease.....	32
7.6. ANIMAL PRION DISEASE.....	33
7.6.1. Scrapie	33
7.6.2. Transmissible mink encephalopathy (TME)	34
7.6.3. Bovine spongiform encephalopathy (BSE)	34
7.6.4. Feline spongiform encephalopathy (FSE).....	35
7.6.5. Exotic ungulate encephalopathy (EUC)	35
7.6.6. Chronic wasting disease (CWD).....	36
7.7. Therapeutic strategies.....	36
VIII. CONCLUSION	39
REFERENCE.....	41

LIST OF FIGURES

Figure

1. PrP processing.....	7
2. The structure of PrP ^C and PrP ^{Sc}	9
3. Subcellular localization and trafficking of PrP ^C	11
4. Two model of conversion of PrP ^C into PrP ^{Sc}	15
5. Regions of the brain affected by prions.....	23
6. Pathogenic event in prion disease.....	27
7. Strategies for treatment.....	38

CHAPTER I

INTRODUCTION

Proteins are the most versatile molecules in the cell. They play an important role in all biological processes. Protein function is assured by its synthesis inside the cell followed by folding into the specific conformational state that is encoded in its sequence. The ability of proteins to fold to their functional states is one of the most remarkable features of biology. However, under some conditions, proteins fail to fold correctly or to remain correctly folded, in living system, and this failure can result in a wide range of disorders. The accumulation of misfolded proteins is a common feature of several neurodegenerative diseases associated with the deposition of proteinaceous aggregates in a variety of organs such as liver, heart and brain. The proteins involved in these conditions are known as prions (proteinaceous infectious particles).¹ Intracellular accumulation of cellular form of prion protein might significantly impair cell function and lead to cytopathology. ² Prion diseases belong to a group of conformational disorders affecting the central nervous system of multiple species. The pathological

mechanism relies upon conversion of physiological cellular proteins into insoluble and toxic conformers accumulating in the brain during the course of the disease. The conversion of largely α -helical proteins into cross- β -plated sheet conformations underlies these disorders.^{3,4} Prions are associated with a group of diseases called transmissible spongiform encephalopathies (TSEs). Those include Creutzfeldt-Jacob disease (CJD), kuru, fatal familial insomnia (FFI), Gerstman-Straussler-Scheinker disease(GSS) in humans, scrapie in sheep, goats, mouflons, transmissible encephalopathy (TME) in mink, bovine spongiform encephalopathy (BSE) in antelopes, feline spongiform encephalopathy (FSE) in domestic cats, exotic ungulate encephalopathy (EUC) in nyala and greater kudu, and chronic wasting disease (CWD) of mule deer and elk.⁵

CHAPTER II

PRIONS

Prions are small, infectious pathogens composed of aggregated, abnormal forms of a protein encoded in the host genome. The protein adopts two forms: normal and infectious. To date, no detectable nucleic acid and no virus-like particles have been associated with prions.^{6,7}

2.1. Discovery

The unusual properties of the infectious agent became the focus of attention beginning in the 1960s. Gibbon, Hunter, Griffith and Levine observed that the agent causing scrapie and Creutzfeldt-Jacob disease is resistant to ultraviolet radiation and consists solely of proteins. In the late 1970s and early 1980s other researchers found that scrapie agent was insensitive to proteolytic digestion.⁸ In 1982, Stanley Prusiner and coworkers first isolated and characterized a putative infectious agent from the

scrapie-tainted brain tissue and proposed the "prion only hypothesis". This model postulated that the pathogenic agent causing transmissible spongiform encephalopathies in human and animal, consists mainly or entirely of PrP^{Sc}, a conformational variant of the constitutively expressed host glycoprotein PrP^C without any encoding nucleic acid. The information for prions is enciphered in the structure of the pathological isoform.^{6,7} The isolation and investigation of the Prnp gene expression in 1985 showed that prion protein was present not only in infected animals, but also in healthy animals.⁹ Moreover, in the absence of cellular expression of PrP^C, even when nonneuronal tissue contains dense deposit of PrP^{Sc} neuronal death does not occur.¹⁰ Conformational change of the cellular form of prion protein to a pathological, disease associated form is considered central to pathogenesis and formation of the infectious agent or prion.^{11, 12} The conformational change of PrP^C into PrP^{Sc} results in a fundamental change of its biophysical properties.⁴

Although PrP^{Sc} is the only known component of the prion particle, these pathogens share some phenotypic traits with viruses. Therefore, some scientists reject the prion protein hypothesis and propose other theories. The most popular is the virino hypothesis which postulates that scrapie agents are a molecular chimera composed of host encoded shell-protein and a nucleic acid.^{5, 13} However, several main features distinguish prions from viruses. First, there is no evidence for nucleic acid within a prion particle, whereas viruses are composed of a nucleic acid genome which serves as the template for the synthesis of progeny virus. Second, PrP^{Sc} encoded by a chromosomal gene, is the only known component of the prion, whereas viruses are composed of

nucleic acid, proteins and often other constituents. Third, viruses exist in a single form with distinct ultrastructural morphology; prions can exist in multiple molecular forms. Fourth, prions are non-immunogenic in contrast to viruses.^{7, 13} Moreover, prions encipher strain-specific properties in the tertiary structure of PrP^{Sc} in contrast to pathogens with nucleic acid that encode these properties in genes.¹⁴ The data from numerous studies presents evidence against the virino hypothesis.

The “unified theory” proposed by Weissmann suggests that the infectious agent designated apoprion is a molecular chimera composed of the misfolded protein that confers infectivity, and unidentified oligonucleotide that specifies strain characteristics.^{5, 15} According to this model apoprion can mediate the infectious process independently or together with the small nucleic acid within the host, the co-prion.⁵

Accumulated evidence supports the protein-only model of infection. The main evidence is the absence of nucleic acid and the resistance of prion to reagents that can damage or modify genetic material (DNAse, RNAse) and susceptibility of infectivity to agents which destroy protein (trypsin, proteinase K).⁷ Additional evidence for the protein-only model comes from yeast and fungi.¹⁶ Two yeast proteins Ure2p and Sup35 after conformational change can form infectious isoforms ([URE3] and [PSI⁺] in *Saccharomyces cerevisiae*).¹⁷

CHAPTER III

CELL BIOLOGY OF PrP^C ISOFORM

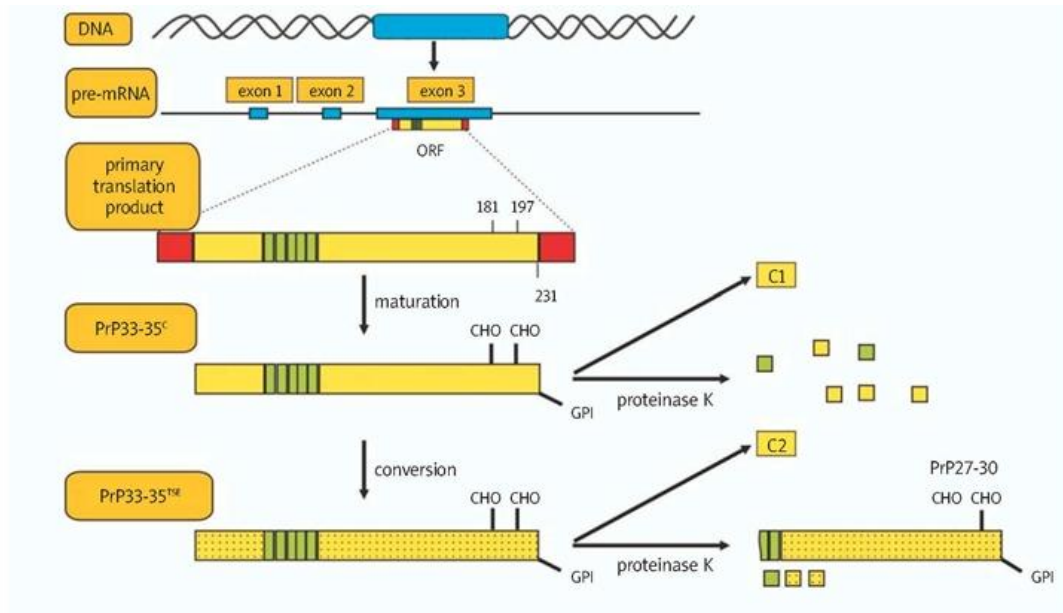
3.1. PrP^C

PrP^C is normal cellular isoform of the protein. It is expressed in the neurons, glia of the brain and spinal cord, in several peripheral tissues and in leukocytes. In the adult central nervous system, PrP^C and its mRNA are widely distributed, with particular concentration in hippocampal and neocortical neurons, cerebellar Purkinje cells, and spinal motor neurons.¹⁸ However, the highest levels of expression are seen in the central nervous system in association with synaptic membranes. PrP^C is expressed during early embryogenesis.¹⁹

3.1.1. Processing

PrP^C is a highly conserved cell membrane sialoglycoprotein. It is encoded by cellular gene located on chromosome 20 in man (PRNP) and chromosome 2 in mouse (prnp).

(Figure 1) Human PRNP gene consists of 2 exons (3 exons in mice, cattle, and some



other species) and an open reading frame located within exon 2 (or 3 in species contain 3 exons).⁸

Figure 1. PrP processing. Normal form of protein PrP^C is synthesized from mRNA within the cell and processed on its way to the cell surface. Two glycans (CHO) at position 181 and 197 are added during the maturation process. Carboxy-terminus and leader sequence are removed before sending to the cell surface. Membrane anchor (GPI anchor) is covalently attached to protein. PrP^C is converted into a pathological isoform. Proteolytic cleavage of PrP^C with proteinase K yield 17 kDa N-terminally truncated form C1. PrP^C is completely degraded. Digestion of PrP^{Sc} yields a peptide designated C2 and a “core protein” designated PrP27-30⁸.

The human PrP^C protein is synthesized as a 253 amino acid polypeptide chain. The first 22 amino acids (signal peptide) are cleaved shortly after translation. During maturation PrP^C undergoes several kinds’ of posttranslational modification including addition of N-linked oligosaccharide chains at two sites, formation of a single disulfide bond, and attachment of the GPI anchor at residue 230, which facilitates glycolipid

linkage of PrP^C to the cell membrane.²⁰ Lack of GPI anchor decrease the infectivity of prions and leads to less neurodegeneration.^{11, 21} PrP^C is the final product which is converted into infectious isoform. PrP^C is expressed equally in the cells of both normal and infected animals.

3.1.2. Structure

PrP^C is usually monomeric in structure, protease sensitive, and linked to cellular membranes through a glycosylphosphatidylinositol (GPI) anchor. It is a four-helix bundle protein containing four regions of secondary structure denoted H1 through H4.²⁰ PrP^C contains about 40 % α helix and little anti-parallel β sheet. (Figure 2) Both N-terminal and C-terminal region of the natural protein consist of around 100 amino acid.²² The C-terminal domain is composed largely of α -helical structure (with three α -helices and a short anti-parallel β -sheet) stabilized by a single disulphide bond linking helices 2 and 3.²² The N-terminal region is very highly conserved in evolution. It contains the octapeptide repeat region between residues 51 and 91 (five repeats of an 8 amino acid sequence) with two tight binding sites for Cu(II) ions and neural cell adhesion molecule (NCAM) that interacts with PrP^C at the neuronal cell surface. Binding of PrP^C protein to Cu(II) ions may regulate cellular copper homeostasis under oxidative conditions. Interaction of PrP^C with NCAM at the neuronal surface may promote recruitment of NCAM to lipid rafts and thereby regulate activation of fyn kinase, an enzyme involved in NCAM-mediated signaling.²³ Mutations in the octapeptide repeat region, resulting in

addition of integral numbers of additional repeats, leads to forms of inherited prion disease.²⁴

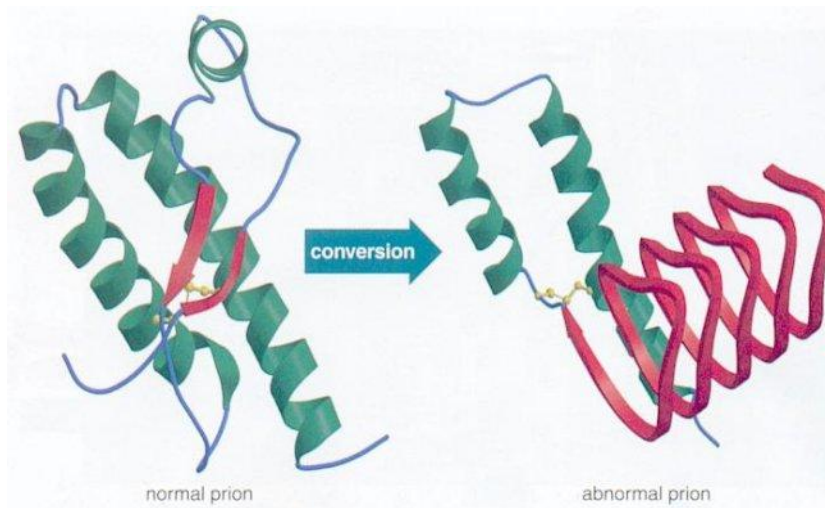


Figure 2. Structure of PrP^C and PrP^{Sc}.

<http://universe-review.ca/F11-monocell.htm>

The prion protein exists in three different topologies: a secreted form (SEC) attached to the outer of the lipid bilayers by a C-terminal GPI, a transmembrane form that spans the lipid bilayer in which the C-terminus is extracellular (CTM), and a transmembrane form in which the amino terminus is extracellular (NTM). The sequence encoding residues 151-165 that forms transmembrane region is highly conserved.²⁵ The transmembrane forms are pro-apoptotic and associated in neurodegeneration in vivo, whereas the secreted form is anti-apoptotic. Many studies suggest that CtmPrP is a main pathogenic form associated with neurodegeneration.²⁶

3.1.3. Function

Although the normal biological function of PrP^C is still unknown, PrP^C is implicated in diverse activities. Possible functions include: neuronal neurite outgrowth and synapse formation, differentiation of neural stem cell, interaction with signal transduction pathway, neuritogenesis, neuronal survival via pro- or anti-apoptotic functions, synaptogenesis, long-term memory formation, development and activation of T cells, modulation of phagocytosis of leukocytes , and altering leukocytes recruitment to the sites of inflammation.^{27, 28, 29} Knockout experiments suggest expression of PrP is not crucial to the survival of organism.³⁰

3.1.4. Cellular trafficking

PrP^C protein is synthesized first in the endoplasmatic reticulum, matured in the Golgi apparatus, and carried in its mature form to the cell surface usually anchored by GPI moiety.³¹ (Figure 3) Generation of PrP^{Sc} occurs after arrival of PrP^C at the cell surface. PrP^C at the surface is predominantly located in specialized detergent-resistant microdomains (DRM) known as lipid rafts. PrP^C may be internalized and processed through the endosomal-lysosomal system, which may also be substrate for their interaction.^{8, 31} PrP^C can be subjected to endocytosis and either recycled to the plasma membrane via recycling endosomes or degraded in lysosomes. PrP^C may also be transported through the membrane as multivesicular-bodies derived exosomes.³² Small membranous particles can be released from multivesicular bodies and fuse with the

membranes of other cells, providing a potential mechanism for the spread of infectivity between cells.²⁷

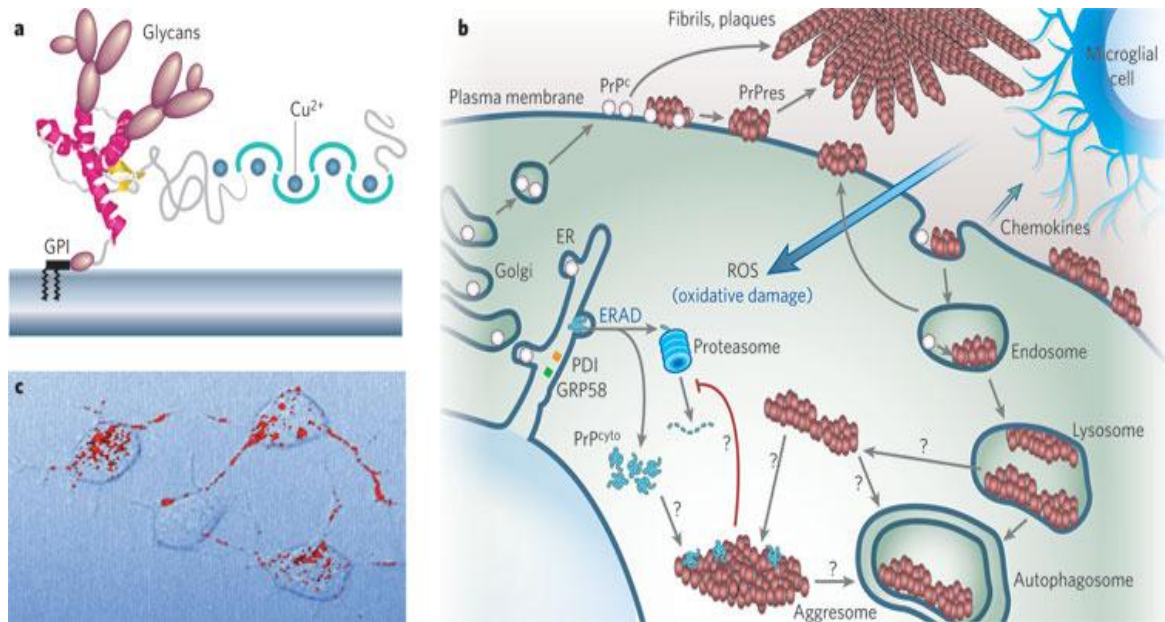


Figure 3. Subcellular localization and trafficking of PrP^C. a) PrP anchored to plasma membrane. b). Cellular trafficking of PrP. c). Neuronal cell with fluorescently tagged PrP^{Sc}.²⁷

http://proxy.ulib.csuohio.edu:2319/nature/journal/v443/n7113/box/nature05294_BX1.html

In central and peripheral nervous system neurons, fast anterograde and retrograde axonal transport of PrP^C facilitating movement to and from neural extremities have also been detected.^{2, 8} Up to 10% of newly synthesized protein might be retrogradely transported from the endoplasmic reticulum to the cytosol, where it undergoes degradation.

3.2. PrP^{Sc}

The "Sc" superscript is used to designate the scrapie-like isoform of PrP since all of the known prion diseases of mammals involve aberrant metabolism of PrP similar to that observed in scrapie. PrP^{Sc} is derived from PrP^C by a post-translational mechanism and no covalent differences between both forms have been demonstrated. PrP^{Sc} acts as a template which promotes the conversion of cellular form. The difference between PrP^{Sc} and PrP^C lies in their conformation and the state of aggregation.²² The same primary amino acid sequence of PrP^{Sc} corresponds to that encoded by the PrP gene but its secondary structure is dominated by beta conformation. PrP^{Sc} is composed of about 30% α helix and 45% β sheet.³³ (Figure 2) Enhanced β sheet content accounts for its innate amyloidogenicity with strong tendency to self-aggregation and fibril formation. Infectious forms of PrP vary and are poorly defined structurally, but are most commonly multimeric (aggregated) and more protease-resistant than PrP^C.³⁴ PrP^{Sc} is able to replicate and propagate itself by transferring its altered conformation to the endogenous PrP^C, a cell surface-enriched protein that becomes partially resistant to proteases and accumulates in plaques in the brain. PrP^{Sc} is partially hydrolyzed by proteases to form an infectious fragment designated PrP²⁷⁻³⁰, while PrP^C is completely degraded under the same condition. Proteinase K digestion of PrP^{Sc} removes a variable number of N-terminal aminoacids (up to around residue 90). N-terminus can be cleaved anywhere from residue 74 to 102. However, a protease sensitive disease associated transitional form has also been described.³⁵

The protease resistant central core of PrP^C designated PrP27-30 was first discovered as a protein copurifying with scrapie infectivity. It was found in the amyloid plaques in brains from humans and animals with putative prion diseases (GSS, vCJD patients and some kuru patients). This form was absent in other disorder such as Parkinson or Alzheimer's disease.⁵ The core protein ranges in size from 27 to 30 kDA. Its tertiary structure is probably distinct from the tertiary structure of normal protein. It doesn't have GPI anchor, cysteine bond and glycosylation sites. It polymerizes into rod-shaped structures with high content of B sheet which are indistinguishable structurally from many purified amyloids.³⁶

Infectious isoform is also resistant to heat, radiation, and formalin treatment commonly used to destroy viruses and other pathogens.²³ PrP^{Sc} accumulates, whereas PrP^C turns over rapidly. The patterns of PrP^{Sc} accumulation in brain are distinct from the distribution of PrP^C.³⁶

PrP^{Sc} accumulate in amygdale, especially in the medial habenular nucleus, the medial septal nuclei, and the diagonal band of Broca.³³

CHAPTER IV

CONVERSION OF PrP^C INTO PrP^{Sc}

Conversion of PrP^C into PrP^{Sc} is the central event in the pathogenesis of transmissible prion diseases.¹¹ Conversion of PrP^C into PrP^{Sc} is conformational rather than covalent. Two isoforms are chemically indistinguishable, have the same primary amino acid sequence and probably the same posttranslational additions. The main difference between PrP^C and PrP^{Sc} seems to be conformational, with PrP^{Sc} having a higher β -sheet content and being multimeric.⁵ PrP^{Sc} is derived from PrP^C by a post-translational modification. Isoform PrP^{Sc} acts as a template that promotes the conversion of PrP^C to PrP^{Sc}. Specific direct binding between PrP^C and PrP^{Sc} precedes the conversion of PrP^C to the PrP^{Sc} conformation.²⁰ The molecular mechanism of that process is still unknown. The experimental data suggest that the conformational alteration could be induced by a yet unidentified event, which occurs spontaneously or by contact with external prions, or by awakening of silent prions.² There are two proposed competing mechanisms of

conversion PrP^{C} to PrP^{Sc} . (Figure 4) The refolding model was originally proposed by Griffith and expanded by Pruisner. It suggests that interaction between externally introduced PrP^{Sc} and cellular PrP^{C} and mixing of those monomers leads to the formation of PrP^{C} - PrP^{Sc} heterodimer which in turn rapidly converts to PrP^{Sc} homodimer.

The seeding mechanism was proposed by Griffith and expanded by Gajdusek and Lansbury. It suggests that the normal and infectious forms of the prion protein are in equilibrium. PrP^{Sc} oligomer or polymer acts as a "bad seed." It can recruit more PrP^{Sc} , increase replication of the infectious agent and eventually aggregate to form amyloid.

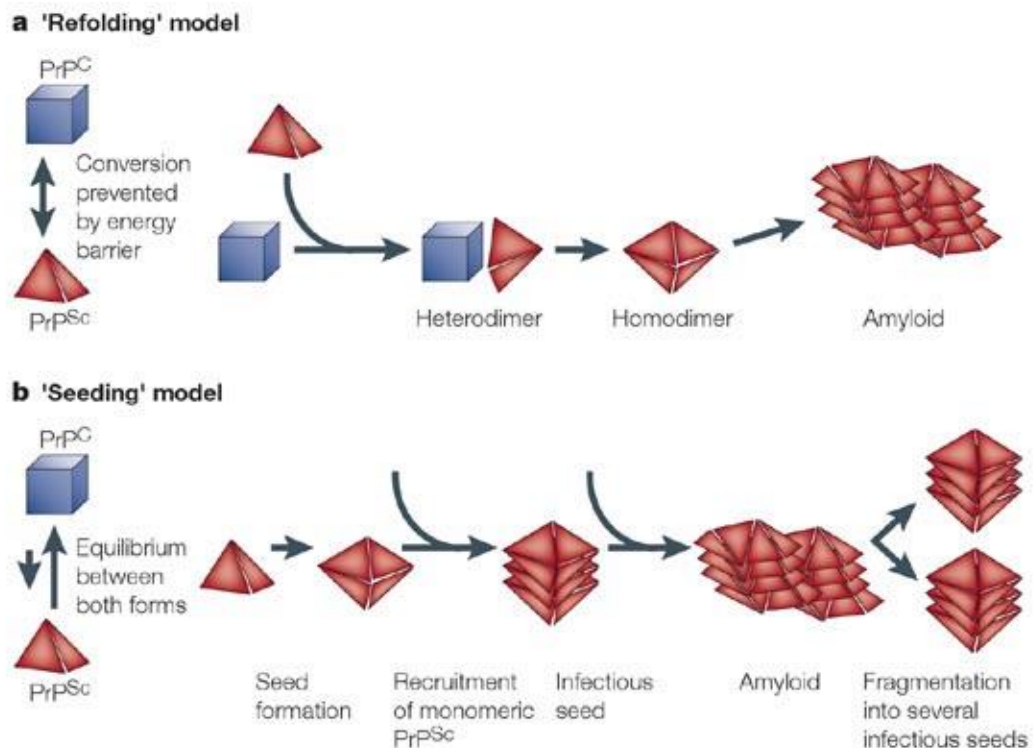


Figure 4. Two model of conversion of PrP^{C} into PrP^{Sc} .

In the infectious manifestation of prion disease, extracellular PrP^{Sc} interacts with PrP^C in lipid rafts on the plasma membrane or in the endocytic organelles, catalyzing its conversion to PrP^{Sc}. Once formed, PrP^{Sc} is metabolically stable and accumulates in intracellular organelles, perhaps endosomes and lysosomes, which may be the site where the N-terminus of the protein is proteolytically cleaved. In familial prion disorders, conversion to PrP^{Sc} is induced by a mutation in the amino acid sequence of the protein. Mutant PrP^C is converted spontaneously via a series of biochemical intermediates, the earliest of which is a PI-PLC-resistant form generated in the ER and delivered to the cell surface. ¹⁸ Although, at least 15 PRNP polymorphisms are known, only methionine or valine at codon 129 is clearly influential in all disease form. ²

CHAPTER V

PRION STRAINS

One of the many remarkable features of prions is the existence of distinct strains. They share the same PrP sequence (identical primary structure) but lead to distinct disease-associated pathology in the brain. These pathological changes include gliosis, spongiosis, neuronal vacuolation, and diffuse versus plaque-like PrP deposits. Approximately 20 strains of scrapie agent have been isolated from sheep and goats affected with clinical scrapie.⁵ The same strain can be isolated from different hosts and the same host can be infected with different strains. Strains of prions have been defined by incubation times and the distribution of neuronal vacuolation.³⁷ Several main features distinguish prions strains. Distinct strains are distinguished by their physical and chemical differences, and by differences in biological properties on transmission to laboratory animals.³⁸ Multiple TSE strains have different incubation time and lesion

profiles upon serial transmission in congenic hosts. Different prion strains target distinct cells in the brain and are localized in different cellular compartments. Distinct strains induce morphologically diverse aggregates ranging from small deposits to large amyloid plaques.³⁹ All this species-specific change in the PrP^{Sc} molecule is accompanied by an alteration in the pathogenicity of the prion.⁴⁰ The existence of biologically different strains of scrapie agent is still the strongest argument against the protein-only nature of the scrapie agent. However, the recent studies suggested that the properties manifested by prion strains such as incubation times and neuropathology profiles are encoded by different conformation of PrP^{Sc} and glycosylation patterns.⁴¹ Such PrP interactions may be most efficient if the interacting proteins are not only of the same sequence but have similar conformational preferences and glycosylation. The experimental data provided by study of transmission of two different inherited human prion diseases to mice expressing a chimeric MHU2M PrP transgene demonstrate that strain-specific information is enciphered in the tertiary structure of PrP^{Sc}.⁴² These strains can be distinguished by differing physiochemical properties of the accumulated PrP^{Sc} in the brains. Variations in tertiary structure of PrP^{Sc} probably correlate with differing surface exposures of the protein, and account for differences in cleavage sites with protease digestion.⁴³ The difference in molecular size of two fragments (HY, DY) after protease treatment was shown to be due to different sites of proteolytic cleavage at the NH₂ termini reflecting different tertiary structures.²⁰ Moreover, the existence of variant CJD associated with PrP^{Sc} glycoform ratios distinct from those seen in classical CJD strongly support the protein-only hypothesis and suggest that strain variation is

encoded by combination of PrP conformation and glycosylation. Only a limited number of different PrP^{Sc} conformations that are highly stable will be permissible thermodynamically and will constitute the range of prion strains seen. Different prion strains can profoundly affect susceptibility of a particular species to prions from another species.⁷

CHAPTER VI

SPECIES BARRIER

Transmission of prion diseases between different mammalian species is restricted by a “species barrier”. Transmission of prions from one species to another is generally inhibited when compared to subsequent passage in the same host species. The strain of prion, the difference in PrP sequence between the prion donor and recipient and the species specificity of unknown factor that binds to PrP^C and facilitates PrP^{Sc} formation, contribute to the species barrier.²⁰

Prion diseases can be transmitted between species by dietary exposure (eating of food contaminated with prion protein) or inoculation.² Iatronic routes include the use of inadequately sterilized surgical and medical equipment because prions resist conventional sterilization. In humans, transmission of the vCJD agent via blood transfusion has been reported.⁴⁴ The disease can be transmitted experimentally by intravenous, intracerebral or intraperitoneal inoculation. Animals infected experimentally with prion diseases are not contagious to humans.²

CHAPTER VII

PRIONS DISEASES

Transmissible spongiform encephalopathies (TSEs) or prion diseases are multifaced degenerative disorders in humans and animals. They originate spontaneously, genetically or by infection.⁵ The term spongiform refers to the characteristic sponge shape lesions that are found in the brain of infected individuals. The key event in prion disease is the conversion of the α -helical, cellular isoform of the prion protein to the insoluble, β -sheet-rich disease-causing isoform.^{3,4} TSEs attack the central nervous system leading to progressive spongiform degeneration of brain tissue. The main clinical features include consequent dementia, motor dysfunction, paralysis and mortality. All recognized prion diseases are invariably fatal. Neither humoral nor cellular immunological responses have been detected in response to prion diseases because PrP^{Sc} is very similar to host protein.⁴⁵ Although there are many morphologic and pathophysiologic similarities between TSEs and other progressive encephalopathies,

such as Alzheimer and Parkinson disease, they are unique in that they are transmissible by inoculation or ingestion of prion-contaminated material.

7.1. Spreading of disease

In most TSEs, infectivity and disease associated, the conformationally altered form of PrP first accumulates in the lymphoreticular and secretory organs. Prion transport and replication require follicle associated epithelium, M cells, follicular dendritic cells, tinged and dome body macrophages. Supporting role have B cells and complement system. Within days after oral exposure prions may penetrate the intestinal mucosa probably through microfold cell and Peyer's patches. Infectivity appears in the spleen mainly in follicular dendritic cells and lymph nodes. Infectivity is associated with B- and T-lymphocytes and with the FDC containing stromal fraction.⁴⁶,⁴⁷ Although FDC are the site of prion propagation in the spleen, some evidence such as neuroinvasion without FDCs suggest the existence of other peripheral cells that can replicate the prion.⁴⁸ Tissues of the lymphoreticular system are sites in which prions accumulate and replicate. A significant role is played by gut-associated lymphoid tissue and draining lymph nodes.⁴⁹ Myeloid dendritic cells mediate transport within lymphoreticular system. However, Infections then spread to the peripheral nervous system, leading eventually to neurodegeneration, accompanied by widespread accumulation of PrP^{Sc}. Prions are transported to the brain primarily by peripheral nerves. Prion diseases affect different parts of the brain. The main parts include cerebral cortex, thalamus, cerebellum and brain stem. (Figure 5)

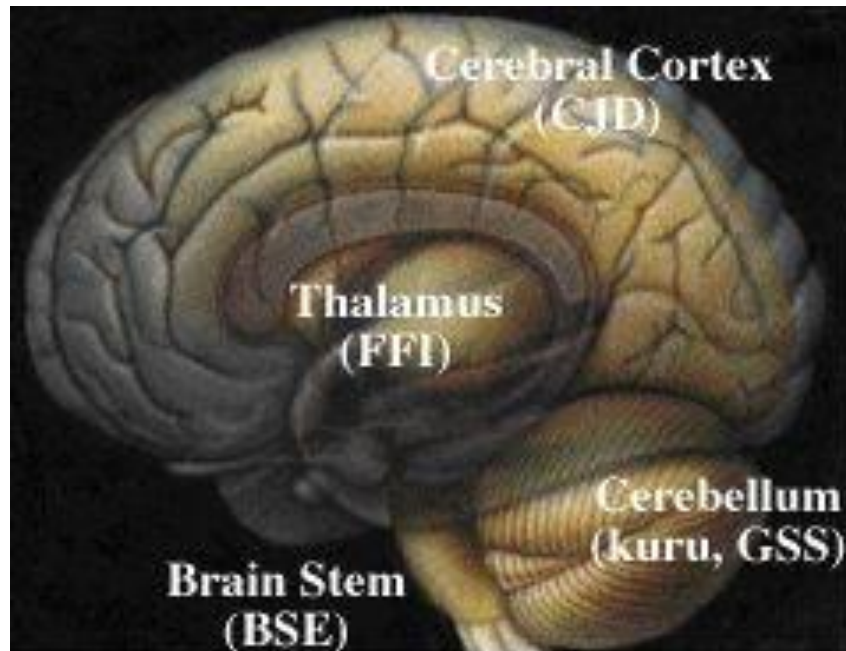


Figure 5. Regions of the brain affected by prions.

<http://nobelprize.org/.../1997/illpres/brain.html>

Morphological analysis shows that first, neurons develop small, round or oval ($\leq 10 \mu\text{m}$) intracytoplasmic vacuoles. With continuous vacuolization, cortical neurophils develop a spongy looking form. Spongy degeneration especially in the second cortical layer is commonly seen in various neurodegenerative diseases.^{2, 50} PrP^{Sc} can also accumulate in astrocytes and microglia. Infection can next disseminate within the central nervous system and spread eventually to peripheral sites such as muscle.⁵¹ The vagus and splanchnic nerves are paths for the initial spread to ganglia and to the CNS. Infection of muscle may happen via neural pathways, for example, efferent projection of motor units to neuromuscular junction and postsynaptically into muscle fibers. Infectivity has been found in some skeletal muscle of mice experimentally infected with

ME7 or RML prions, although prions have not been detected in muscle of BSE-infected animals and scrapie.⁵²

7.2. Type of prion diseases

Prion diseases are divided etiologically into inherited, acquired (iatrogenic) and sporadic forms.

10-15% of recognized prion disease is an inherited disorder associated with coding mutations in PRNP gene. More than 30 mutations of the PrP gene have been identified in families suffering from inherited human prion diseases. Researchers suggest that the mutations in the PrP gene lead to production of unstable PrP^C protein that can spontaneously convert into the abnormal isoform.⁵³ Pathogenic mutation includes point and insertional mutation. Point mutations occur in the C-terminal domain of PrP and lead to aminoacid substitution. Insertional mutation encoding additional integral copies of an octapeptide repeat that is present in five copies in the normal protein occur in the N-terminal domain of the PrP^C. Not all PRNP mutation-associated disease in humans is transmitted. The inherited prion diseases can be further subdivided into 3 phenotypes: fCJD, GSS, FFI.²⁰

The acquired prion diseases include CJD, variant CJD, BSE, scrapie and kuru. Infections arise from accidental exposure to human prions through participation in cannibalistic feasts or medical procedures (i.e. treatment with pituitary derived hormones, tissue grafting, corneal transplantation, and neurosurgery).⁵⁴

Sporadic form is the most common and makes up around 85% of all diagnosed human prion disease. CJD and FFI occasionally occur in people who have no family history of the disease and no known exposure to infectious prions. The possible causes are: spontaneous somatic mutation of PRNP, spontaneous transition of PrP^C into PrP^{Sc} or unidentified environmental prion exposure. The sporadic CJD occurs in homozygote at codon 129 encoding valine or methionine. Heterozygotes do not develop this disease.

Polymorphism of PRNP at codon 129 which is a major determinant of genetic susceptibility to and phenotypic expression of prion disease is associated with four different types of human infectious protein. PrP^{Sc} type 1 and 4 is observed in MM individuals, type 3 in MV or VV and is predominantly associated with at least one V allele. Type 2 has been detected in any PRNP codon 129 genotype. PrP^{Sc} type 1-3 is associated with sporadic, iatrogenic and classical CJD. Type 4 human PrP^{Sc} is predominantly associated with vCJD.⁵⁵

7.3. Protein aggregation and disease

PrP^C and its pathological isoform bind to each other forming aggregates. It is still not clear if these aggregates are the cause of the cell degeneration or a side effect of disease. There are a few hypotheses that developed during the past 15 years to explain the relationship between protein aggregation and disease. The first hypothesis states that the formation of aggregates is a direct effect of disease. Conversely, the “amyloid model” states that human neurodegenerative disorder causes fibrillar- protein deposition.³⁴ However, there are many evidences that argue against both hypotheses.

Amyloid plaques were found in the brain of normal 70 years old individuals. Protein aggregates are not always coupled to disease and some disorder such as GSS do not show fibrillar deposits of abnormal protein. Moreover, the amount and the size of amyloid plaques examined in postmortem brain do not correlate with severity of symptoms at the time of death ⁴⁶. Therefore, researchers proposed the third revised model to explain the qualitative correlation (protein deposition are not quantitatively correlated with disease) between the disorder and protein aggregation. This hypothesis states that unknown agent initiates both protein aggregation and the disease. This model explains extreme situation described above and suggests the existence of “prevalent deposits but little or no disease or conversely severe disease with little or no deposits”. ³⁴

7.4. Mechanism of cell death

Death of the nerve cell underlies the clinical symptoms of prion diseases. ⁸ However, the cellular pathways and the molecular mechanism that cause the neuronal damage are still not entirely clear. Early studies argued that the neuronal damage might be the effect of loss of the normal function of PrP^C or gain of toxic property of PrP^{Sc}. ²

In the absence of normal form of protein neuronal loss does not occur. The disease is prevented when neurons cannot produce normal form of protein even in the presence of dense deposits of abnormal isoform. ⁵⁶ Moreover, early neuronal damage is reversed when neuronal PrP^C is depleted in mice with ongoing prion infection. ⁵⁷ Hence, conversion of normal protein to infectious isoform is central to pathogenesis.

Therefore, the main question is whether PrP^{Sc} is itself directly the cause of the neuronal damage. In vitro experiments show that PrP^{Sc} and short PrP peptides are neurotoxic. However, prion diseases occur with high levels of PrP^{Sc} in brain with or without clinical symptoms. There are examples of prion infection in which the level of pathological isoform is very low or even not detectable.⁵⁸ According to the latest studies prions themselves are not directly neurotoxic. Toxic species derived during prion propagation produced directly from PrP^C or as a PrP^{Sc} precursor, are probably responsible for neuronal damage.²⁷

Molecular and cellular pathways leading to neurodegeneration remains still unclear. However, recent studies suggest that the pathway leading to neuronal loss include ubiquitin-proteasome and endosomal system, oxidative stress, regulated activation of complement, synaptic alterations and dendritic atrophy.² (Figure 6)

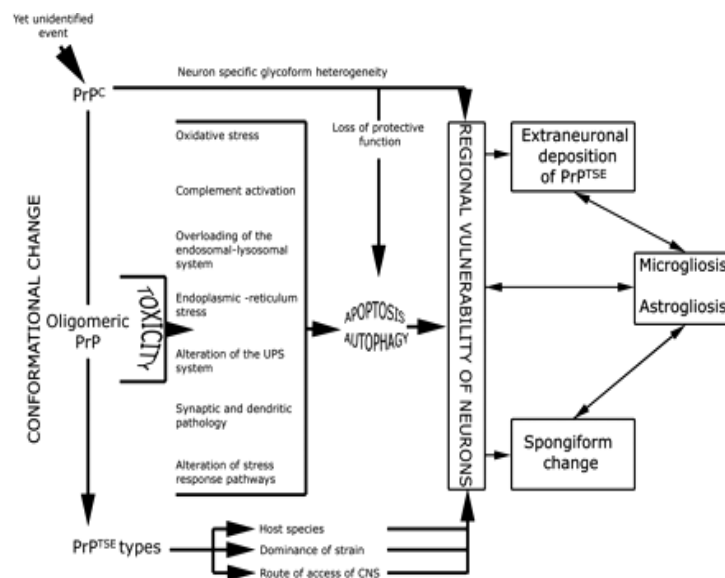


Figure 6. Pathogenic events in prion disease. <http://ajp.amjpathol.org/cgi/content->

[nw/full/172/3/555/F3](http://ajp.amjpathol.org/cgi/content-nw/full/172/3/555/F3)

7.5. Human prion diseases

Human prion disease has been classified into: Creutzfeldt-Jacob disease (CJD), kuru, fatal familial insomnia (FFI), Gerstman-Straussler-Scheinker disease (GSS) and Alpers' disease.⁵ Prion protein causes characteristic lesions in the brain with characteristic spongiform vacuolation, accumulation of amyloid plaques, neuronal cell loss, microglial activation and proliferation of astrocytes. Neuropathologically, these disorders produce a characteristic spongiform degeneration of the brain, as well as deposition of amyloid plaques.⁵⁹ The primary symptom of the human disorders is dementia, usually accompanied by manifestation of motor dysfunction such as cerebellar ataxia, myoclonus, pyramidal or extrapyramidal signs.¹⁸

7.5.1. Creutzfeldt-Jacob disease (CJD)

Creutzfeldt-Jacob disease was discovered by two physicians, Creutzfeldt and Jacob in the 1920s. There are three different forms of CJD: sporadic, inherited and iatrogenic.²²

Sporadic (classical) CJD is a rapidly progressive, multifocal dementia, with two or more myoclonus that affects individuals between 45-75 years. Around 90% of patients die within 12 months. The central clinical feature include: mental deterioration, insomnia, depression, general malaise, fatigue. Damage to the cerebral cortex associated mainly with CJD results in loss of memory and very often visual impairment. Cognitive decline and behavioral disturbance are invariable features.^{54, 56} Myoclonus

and cerebellar ataxia are recognized in up to 70–80% of cases. Frequent additional neurological features comprise: cortical blindness, pyramidal, extrapyramidal and cerebellar signs, akinetic mutism.

Inherited CJD is caused by the inheritance of a PRNP gene with a mutations encoding most commonly lysine instead of glutamic acid at position 200 (E200K), and asparagines instead of aspartic acid at position 178 (D178N) especially when it is associated with polymorphism in both PRNP genes that encodes valine at position 129 (MM). 10–15% of the cases of CJD are inherited as an autosomal dominant.

About 1% of CJD cases are due to acquired infection. Iatrogenic CJD is characterized by progressive cerebellar syndrome and behavioral disturbance or classical CJD syndrome. It attacks people in different age also young. Most cases are caused by injection of pituitary growth hormone or by implantation of dura mater. Direct incubation time depends on the site of prion inoculation.⁶⁰

Variant CJD is referred to as human BSE, because both disorders are caused by the same prion. This disease was first reported in 1996 in 10 patients. Compared to the classical CJD this variant shows atypical clinical manifestation and slower course. Unlike in CJD, dementia is not the most prominent sign of disease. The early clinical feature include: depression, anxiety, peripheral sensory symptoms, behavioral change, social withdrawal, and in later stage: cerebellar ataxia, chorea, or athetosis. vCJD develops in younger people-age 29; however age at onset 12-74 years is seen. The main cause is 129 MM mutation in PRNP gene.⁶¹There is pericellular PrP deposition in cerebral and cerebellar cortex. vCJD plaques are also seen in tonsil and other lymphoreticular tissue.

Historical connection and many experiments strongly indicate a correlation between the prion strain that causes vCJD and BSE. The glycosylation pattern in mice with vCJD was very similar to the pattern found when the transmission of the BSE prion strain was examined. Lesion profile found in the brain of the mice was identical when an animal was inoculated with BSE or vCJD agent and distinct from all other not BSE related TSE's. The examination of incubation time and survival ratio had the same result. ⁶¹

7.5.2. Kuru

Kuru is an invariably fatal disease that appeared at the beginning of 20th Century among a population living in the Eastern Highlands of Papua New Guinea. Kuru means “laughing death” and was used by Fore people to describe uncontrollable laughter accompanied with this disease. Kuru was transmitted among the members of this group by ritual cannibalism. The disease spread between members of the Fore tribe especially women and children, because they predominantly participated in the feast and ate brain and internal organs of their dead relatives. The principal route of kuru transmission was dietary exposure. The infection could also been transmitted by inoculation with brain or other tissue via cuts, sores or following eye rubbing into the conjunctiva. Intracerebral or optic inoculation of experimental animals manifest clinically as classical CJD, with a rapidly progressive dementia. Peripheral inoculation leads to progressive cerebellar ataxia. ⁵⁶

The mean incubation period of kuru have been estimated to be approximately 12 years but sometimes can exceed 50 years. The mean clinical duration of illness is 12

months, with a range of 3 months to one year after the appearance of symptoms. The course tends to be shorter in children. The prion accumulates mainly in cerebellum. The disease affects both mental and motor function. The central clinical feature is progressive cerebellar ataxia. The symptoms include: muscle tremors, incoordination of movement, inability to hold things, involuntary oscillation of the eyes, difficulty in articulating words, swallowing and finally dementia and death.^{54, 56, 61}

No new cases of kuru have been reported since 1950's when disease reached its peak killing several thousand people. Therefore, it is generally believed that kuru is now extinct.

7.5.3. Fatal familial insomnia (FFI)

This hereditary prion disease was first reported in Italy, in 1986. It is very rare disorder that attacks 1 person per 50 million/year between the ages of 40 and 60 years. The mean duration is approximately 7 to 33 months. Prions affect mainly thalamus in the brain that results in insomnia. The core clinical features include: disruption of the normal sleep-wake cycle, insomnia and sympathetic overactivity. Patients exhibits illusion, hallucinations, poor memory, restlessness, inability to concentrate. Other symptoms include different motor disturbances, hormonal disturbances especially growth hormone, increased heart rate and depression. The cause of FFI is associated with mutation at codon 178 with asparagine instead of aspartic acid (D178N) combined with methionine homozygosity at codon 129. Heterozygous for methionine at codon 129 patients have a longer survival than homozygous.^{54, 61}

7.5.4. Gerstman-Straussler-Scheinker disease (GSS)

This inherited disease was discovered in 1928 in Germany. It is a very rare autosomal dominant disorder that attacks 1-10 individuals per 100 million/year. It is frequently observed in patients at the age of 20-30 and sometimes older. The mean duration is approximately 10 years. The typical clinical features of this disorder are slowly progressive cerebellar ataxia with pyramidal features, beginning in the fifth or sixth decade. Dementia occurs later in a more prolonged course than in CJD. The symptoms include: incoordination of movement, stumbling, dysarthria, difficulties swallowing and speaking, amnesia and finally dementia. In contrast to other inherited prion diseases, it has unique neuropathologic features that consist of widespread, multicentric PrP-amyloid plaques rather than spongiform changes.⁶¹ They are located mainly in cerebellum what results in problems to coordinate body movements and difficulties walking. GSS is associated with several different PRNP mutations. The patients have inherited at least one copy of a mutated *PRNP* gene. Some of the most common mutations are: a change in codon 102 converting proline to leucine (P102L) a change from alanine at position 117 to valine (A117V). The disease is accompanied with homozygosity for a polymorphism at position 129 (MM).⁵⁵

7.5.5. Alpers' disease

Alpers' disease is an extremely rare, fatal prion disease of infants that was first time described in 1930's. This chronic encephalopathy is associated with diffuse

progressive degeneration in the brain cortex. In addition, this disease is characterized by degeneration and fibrosis of the liver. The clinical features include: motor disturbances, partial paralysis, seizures, growth retardation, mental disorders, dementia or early death.^{54, 61}

7.6. Animal prion disease

Animal prion disease includes: scrapie in sheep, goats, mouflons, transmissible encephalopathy (TME) in mink, bovine spongiform encephalopathy (BSE) in antelopes, and cows, feline spongiform encephalopathy (FSE) in domestic cats, exotic ungulate encephalopathy (EUC) in nyala and greater kudu, and chronic wasting disease (CWD) of mule deer and elk. Small animals, like mice, hamsters or bank voles can be infected experimentally.⁵

7.6.1. Scrapie

Scrapie is an example of prototype prion disease that affects sheep and goats. Scrapie has been recognized in Europe for over 200 years and is present in many countries worldwide. The disease was called 'scrapie' based on the characteristic behavior of the sheep. The mean incubation period range from 2 to 5 years. The clinical features of the disease include severe weight loss, incoordination of movement due to loss of motor control, paralysis, dementia, wasting and death.⁵⁴ The disease mainly affects brain of the animal leading to the formation and accumulation of plaques, vacuoles and amyloid protein deposits. The mode of natural transmission remains still

unclear. It can be transmitted from animal to animal in feed that is contaminated with infected nerve tissue. It can also be transmitted by injection of brain tissue.^{54,61}

7.6.2. Transmissible mink encephalopathy (TME)

This disease was first reported in 1947 in the United States, when it killed thousands of minks. It affects both female and male minks usually more than one year old. The mean incubation period is 7 months. The animals die within a week or sometimes a month after the appearance of symptoms. The most important clinical features of the disease are behavioral changes.⁶¹ The animals are increasingly nervous, aggressive, experience difficulty in eating and they show incoordination of movement. In the later stage of the disease minks become sleepy, inactive, unresponsive and isolate themselves. The main cause is dietary exposure for example eating contaminated tissue such as meat of scrapie-infected sheep. It has been suggested that disease can be transmitted between foxes through biting. Cattle can be also a source of infection. Experimentally, TME is produced by intracerebral injection of brain material from infected sheep or dietary exposure.⁵⁵

7.6.3. Bovine spongiform encephalopathy (BSE)

Mad cow disease first time described in 1987 in British cows has caused the deaths of almost 200000 cattle. Its origin appears to have been cattle feed that contained brain tissue from sheep infected with scrapie. The mean incubation period is

4-5 years.⁶¹ The first symptoms are behavioral changes such as aggressiveness, irritability, excessive response to different stimuli like light and sound. The clinical features comprised incoordination of movement, running and jumping in air, high steps, paddling and stumbling. In the last stadium of the disease animals have breathing problems, difficulty in urination and defecation. Animal dies within 1-6 months after the appearance of symptoms.^{54, 61}

7.6.4. Feline spongiform encephalopathy (FSE)

This disease was first described in 1990 in a domestic cat in Bristol. It primarily affects domestic animals and different strains of wild cats. It can be also transmitted to mice. The disease is characterized by sleepiness, failure to respond to light stimuli, papillary dilatation, crawling, muscle tremors.^{54, 55, 61}

7.6.5. Exotic ungulate encephalopathy (EUC)

This disease affects wild ungulates, antilopes, nyalas, gemsboks, oryxes, and greater kudus. It was first time reported in the nyala in 1987. The morphological analysis of the brain of infected animals shows typical spongiform changes. The clinical features include: muscle tremors, incoordination of movement, loss of weight, excessive salivation, licking of lips.⁶¹

7.6.6. Chronic wasting disease (CWD)

CWD is a rare disease that affects elk, mule deer, black and white-tailed deer. It was first time reported in Rocky Mountain elk and the mule deer in 1967. The clinical features include behavioral changes and progressive loss of condition.^{54, 61}

7.7. Therapeutic strategies

All recognized prion diseases are invariably fatal and to date no effective therapy is available. However, the major advances in molecular genetics and in understanding molecular pathogenesis of prion diseases enable preparing effective therapeutics for human and animal prion disease. The general purpose of treatment is to slow progression of the disease not to cure, because of serious damage of the brain that occurs before clinical symptoms.

The main therapeutic strategies are aimed at reducing the expression level of normal protein (down regulation of PrP transcription or translation), and prevent conversion of PrP^C (PrP^C stabilization by binding it with small ligand) to pathological isoform which in turn will attenuate prion replication and prolong survival time. (Figure 7)

To date, several drugs which decrease concentration of PrP^{Sc} and target PrP^{Sc} formation have been described in prion infected cell lines. However, none of them is effective when given around the time of the clinical phase manifestation. The most effective and least toxic drugs include PPS, porphyrins, amphotericin B, and copper.⁶²

Researchers develop active vaccines that markedly delay and prevent brain disease in mice similar to BSE. One of the latest examples is a mucosal prion vaccine composed of attenuated Salmonella strain which express prion protein. Vaccines could prevent animals from becoming infected but their use in humans in order to resist prion infection needs further research.⁶³

The most important and promising progress has been made in immunotherapeutic strategies. The latest discovery is antibodies that bind to pathologic isoform of PrP^C and reduce or inhibit its propagation. By binding with PrP^{Sc} it enable the host immune system to recognize the abnormal prion as an invader and attack them. Transgenic mice expressing antibody against the Tyr-Tyr-Arg amino acid sequence of PrP^{Sc} are protected against peripheral prion infection. Moreover, PrP^{Sc} levels in the spleen of peripherally infected mice were significantly reduced even when antibody where administered during the very high level of infectious isoform.⁶⁴

For both humans and animal using immunotherapeutic strategies could significantly inhibit prion propagation, delay the development of prion diseases, and provide useful screening and diagnostic tool for prion protein.

Recent experiments suggest the role and future importance of RNA interference in treatment of prion diseases. shRNA against PrPC reduce it expression and decrease its conversion to pathological isoform. These anti-PrPC shRNA carried on a lentivector is transcribed in neuronal cell and then processed to form siRNA. siRNA lead to degradation of mRNA by activation of RNA-induced silencing complex. Transgenic mice transfected with anti-PrPC sHRNA showed significant increase in survival time.⁶⁵

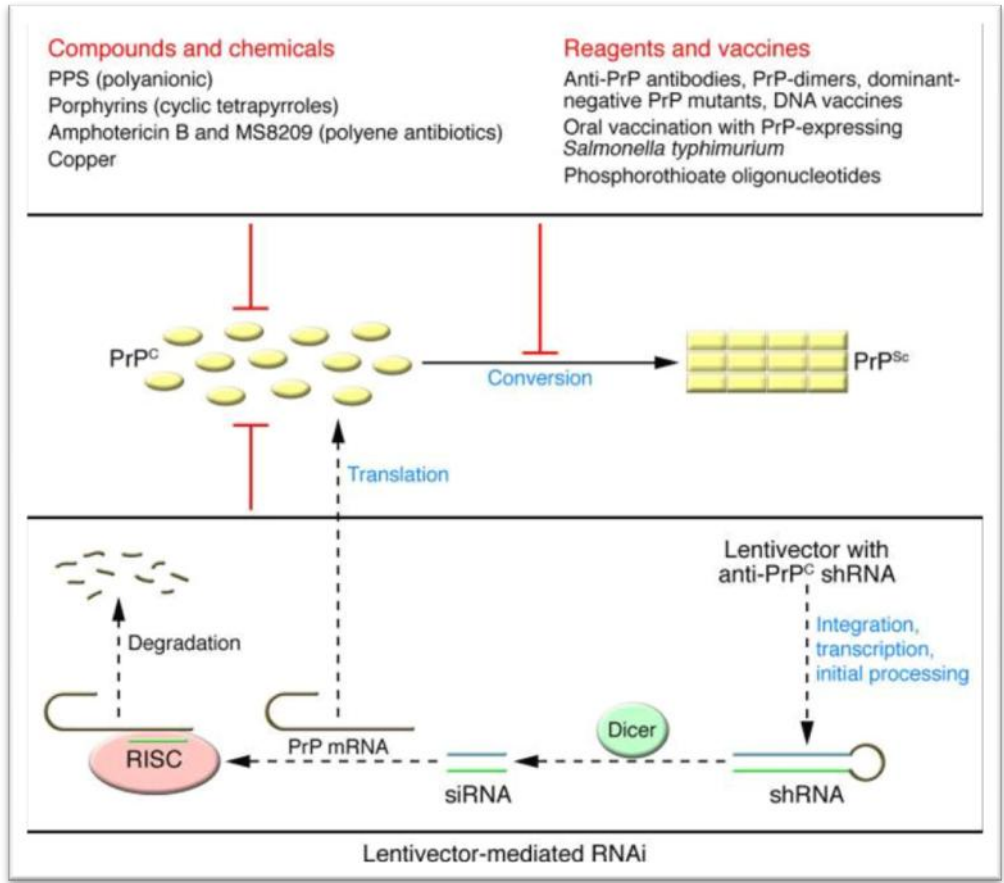


Figure 7. Strategies for treatment of prion diseases.⁶⁶

CHAPTER VIII

CONCLUSION

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of human, animal and yeast caused by an infectious agent designated prion. The "protein only" hypothesis proposed more than three decades ago suggests that the prions are small, infectious pathogens composed of aggregated, abnormal forms of a protein encoded in the host genome. PrP^C plays primary role in neurite outgrowth, synaptic function, oxidative stress defense and long term survival of cerebellar neurons. The key event in prion disease is the conversion of the α -helical, cellular isoform of the prion protein to the insoluble, β -sheet-rich disease- causing isoform. Aggregation of misfolded prion protein into large amyloid plaques and fibrous structures is associated with neurodegeneration. TSEs attack the central nervous system leading to progressive spongiform degeneration of brain tissue. Molecular and cellular pathways leading to neurodegeneration remains still unclear. However, the recent studies suggest that

pathway leading to neuronal loss include ubiquitin-proteasome and endosomal system, oxidative stress, regulated activation of complement, synaptic alterations and dendritic atrophy.²The study of the unusual properties of the infectious agent and pathology of neurodegenerative disorders has been a source of excitement and arguments between the scientists. The major advances in molecular genetics and recent understanding of prion propagation and neurotoxicity allowed deep insight into prion biology. However, many aspects of prion disease still raise controversies and questions for a broad audience of researchers. Understanding the physiological role of PrP, determination of pathological mechanism of neurodegeneration in prion diseases, explaining the relationship between TSEs and other neurodegenerative diseases is a big challenge for the biologist for the next years that will provide the basic for the development of therapeutic modalities.

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