Chapter 20

Subviral Agents and Prions

Chapter Outline

A number of virus-like transmissible agents have been discovered, which do not comply with the classical definition of "virus". These virus-like agents cannot be classified as viruses in the strict sense; nonetheless, they were first considered as "viruses" until their nonviral features were unfolded later. Now, these virus-like transmissible agents are collectively termed "subviral agents." (see Table 1.4).

20.1 SUBVIRAL AGENTS

Subviral agents are composed of three kinds: satellite viruses, viroids, and prions (Table 20.1). These transmissible agents are classified as subviral agents as they are less than a virus in some respects. The first subviral agent is the "satellite virus," which is morphologically indistinguishable from ordinary virus particles, but it depends on another virus, a host or helper virus, for propagation. Therefore, a satellite virus is often called "a parasite of a parasite," as it relies on another parasite, a virus. The second subviral agent are "viroids" found in plants. Viroids are comprised of "RNA only," and are devoid of any protein component. Remarkably, it is a circular RNA molecule itself about only 0.3 kb in length. Nonetheless, the viroid RNA is transmissible and causes pathogenic lesions in plants. The third subviral agent are "prions," which are etiological agents of neurodegenerative diseases, such as mad cow disease. In contrast to the viroids, prions are transmissible agents that are comprised of "protein only," and are devoid of any nucleic acid components.

20.2 SATELLITE VIRUSES

Satellite viruses[1] are mainly found in plants. The replication of a satellite virus depends on a helper virus, while the replication of the helper virus does not depend on the satellite virus. Importantly, no sequence homology is found between host virus and satellite virus, implicating no genetic relatedness of satellite viruses to their hosts.

Satellite viruses found in plants typically possess their own capsid (Table 20.2). For instance, tobacco mosaic virus (TMV) is a helper virus of satellite tobacco mosaic virus (STMV). Unlike TMV, which has a long helical capsid, STMV has a spherical capsid. Satellite tobacco necrosis virus (STNV), the first plant satellite virus, was discovered as a virus-like particle abundantly present in cultured medium of tobacco necrosis virus (TNV), a host virus. Its identity as a satellite virus was revealed by its dependence on TNV for propagation. STNV encodes only one protein, a capsid protein. Its host dependence is specific for TNV. In other words, host dependence of satellite viruses is generally specific. STNV does not encode its own RNA-dependent RNA polymerase (RdRp), a helper function that is presumably provided by TNV.

1. **Satellite virus** It refers to a virus, which depends on the host virus as a helper.

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TABLE 20.1 The Major Features of Subviral Agents

Features	Satellite Virus	Viroids	Prions
Nucleic acid	RNA or DNA	RNA	No
Protein-coding	Yes (capsid)	No	Yes (host)
Protein in particles	Yes (capsid)	No	Yes (PrP)
Helper-dependency	Yes	No	No
Infectivity	Yes	Yes	Yes
Disease	Yes	Yes	Yes

TABLE 20.2 Satellite Viruses in Plants

Plant Satellite Virus	Genome Size (nt)	Protein
Satellite tobacco necrosis virus (STNV)	1239	Capsid (20 kDa)
Satellite tobacco mosaic virus (STMV)	1059	Capsid (18 kDa)
Satellite panicummosaic virus (SPMV)	826	Capsid (18 kDa)
Satellite maize whited line tobacco mosaic virus (SMLMV)	1168	Capsid (24 kDa)

In contrast to the abundance of satellite viruses in plant viruses, only a few satellite viruses are found in animal viruses. Two representative satellite viruses among human viruses are adeno-associated virus (AAV) and hepatitis delta virus (HDV). AAV is a satellite virus of adenovirus (Fig. 20.1), while HDV is a satellite virus of hepatitis B virus (HBV) (see Fig. 23.13). AAV depends on adenovirus for its genome replication (see Fig. 10.4), while HDV relies on HBV for its envelope glycoprotein (ie, HBsAg).

20.3 VIROIDS

Viroids are the smallest infectious pathogens known, comprised solely of a short circular RNA without protein coats. Viroids are plant pathogens with economic importance. Viroid genomes are extremely small in size, only about 300 nucleotides. Viroids have been found in agricultural products, such as potatoes, tomatoes, apples, and coconuts. Several viroids-induced diseases are of considerable economic importance (Table 20.3). For example, yield losses can be high in potatoes infected with potato spindle tuber viroid (PSTVd), citrus infected with Citrus exocortis viroid, coconut palms infected with Coconut cadang-cadang viroid (CCCVd), and avocado infected with Avocado sunblotch viroid (ASBVd) (Fig. 20.2). Exclusion or eradication of infected material is the most effective means of controlling viroid diseases. Viroid infections are transmitted by cross-contamination following mechanical damage to plants as a result of horticultural or agricultural practices. Some are transmitted by aphids and they can also be transferred from plant to plant by leaf contact.

Viroids, the first known representatives of a new domain of "subviral pathogens," stand out in many respects. First, viroids are the only infectious agents that lack protein components such as capsids. In general, the role of the viral capsid is the protection of the viral genome from degradation. Second, the viroid has a circular RNA genome, unlike most RNA viruses. Moreover, the viroid is not included in the Baltimore classification (see Fig. 1.9). The circular configuration confers resistance to exonucleases on the viroid RNA, a feature that makes the protein coat dispensable. Notably, human HDV also has a circular RNA genome (see chapter: Hepatitis Viruses); hence, HDV is often called

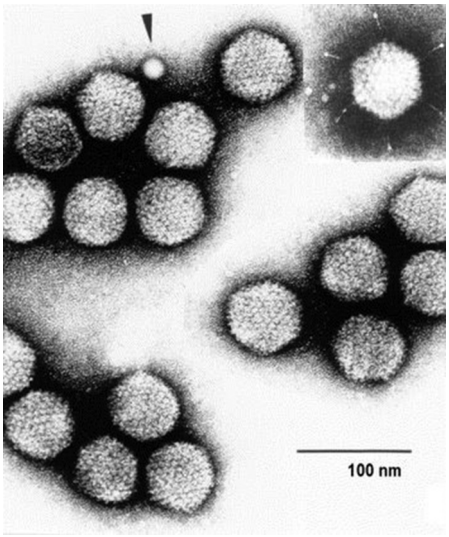


FIGURE 20.1 Electron micrograph of adenovirus and its satellite virus, adeno-associated virus (AAV). Electron microscopic image of AAV particles. AAV particle (arrow head) is seen in the midst of adenovirus particles, a helper virus. Note that the morphology of AAV, a satellite virus, is distinct from that of adenovirus particle, a host virus.

TABLE 20.3 The Main Features of Plant Viroids

Viroid	Abbreviation	Size (nt)
Potato spindle tuber viroid	PSTVd	356–360
Coconut cadang-cadang viroid	CCCVd	246, 247
Tomato apical stunt viroid	TASVd	360
Apple scar skin viroid	ASSVd	360
Avocado sunblotch viroid	ASBVd	246–250

"viroid" in animals. Intriguingly, both viroids and HDV possess a rod-shaped RNA genome, in which about 70% of bases are base-paired (Fig. 20.3). Third, viroids do not code for any proteins. The genome size is indeed too small to encode any functional protein. Fourth, despite the small genome, a helper virus is not required for viroids. In contrast, HDV encodes one viral protein (ie, delta antigen) and relies on the envelope protein of HBV for the virion assembly (see Fig. 23.13). Hence, viroids are not satellite viruses. Overall, the viroid does not fit into the classical definition of "virus," although it is a "submicroscopic infectious agent" having a nucleic acid genome.

Then, how does a viroid replicate its RNA genome? The so-called rolling-circle mechanism has been proposed to account for the RNA genome replication of the viroids (Fig. 20.4). Note that the rolling-circle mechanism has been proposed for herpesvirus and bacteriophage lambda (see Box 10.2). According to the mechanism, by using the infected circular RNA (+), the multimeric linear RNA (−) is synthesized. Then, the multimeric linear RNA is converted to a circular RNA (−) following cleavage to a genome-length. Such yielded circular RNA (−) is then used as a template for the synthesis of the (+) strand RNA.

At least three enzymatic activities are required to fulfill the rolling-circle mechanism for the viroid replication. First, RdRp is needed to carry out the RNA synthesis. Since hosts do not code for RdRp and the viroids do not code for any protein, the identity of the RdRp seems enigmatic. Intriguingly, the viroid RNA replication is α-*amanitin*-sensitive, which potently inhibits cellular RNA polymerase II. An interpretation is that RNA polymerase II, which normally utilizes a DNA template, uses the viroid's RNA as a template. In other words, the viroid subverts the host DNA-dependent RNA polymerase to replicate its RNA genome. It is believed that RNA polymerase II recognizes the

FIGURE 20.2 Potatoes with spindle tuber symptoms. PSTVd-infected tubers may be small, elongated, from which the disease derives its name, misshapen, and cracked.

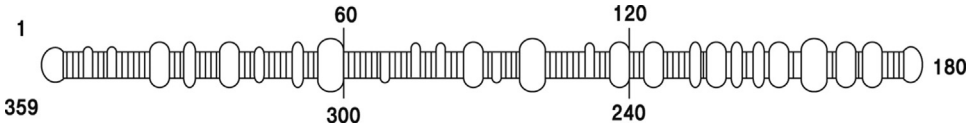


FIGURE 20.3 A secondary structure of plant viroids. A rod-shaped secondary structure of a circular RNA genome of PSTV is drawn. The numbers indicate the nucleotide number of the PSTV genome. The base-pair is denoted by lines.

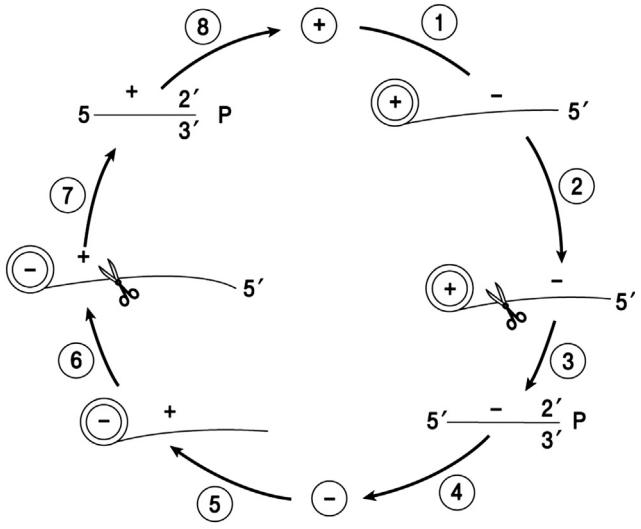


FIGURE 20.4 A rolling-circle mechanism of viroid replication. (Steps 1−2) Multimeric negative-strand RNA is synthesized by using infected viroid RNA (+) as template. Here, the infected viroid RNA is defined as positive-strand (+). (Step 3) The multimeric RNA is cleaved. The cleavage site is denoted by a scissor. P denotes the 2′, 3′ phosphate group at the cleavage site. (Step 4) The unit length RNA is then circularized to an antigenomic RNA (−). (Steps 5−8) The synthesis of positive-strand RNA by using antigenomic RNA is carried out, as steps 1−4.

rod-shaped secondary structure of the viroid RNA, mimicking the double-strand DNA, as a template. It is intriguing that the viroids explore cellular DNA-dependent RNA polymerase for their RNA replication, although the notion has not been formally proven.

Another enzyme required for the viroid RNA replication is an endonuclease that cleaves the multimeric linear RNA to a genome-length and also an RNA ligase that circularizes the cleaved genome-length RNA. Surprisingly, these two enzyme activities are attributed to the viroid RNA itself. In fact, the *ribozyme* (ie, RNA enzyme) encoded in the viroid RNA catalyzes these two enzymatic reactions. Specifically, the secondary structure of viroid RNA, dubbed "hammer head," carries out the sequence-specific endonuclease reaction (Fig. 20.5). Following the cleavage, the rod-shaped

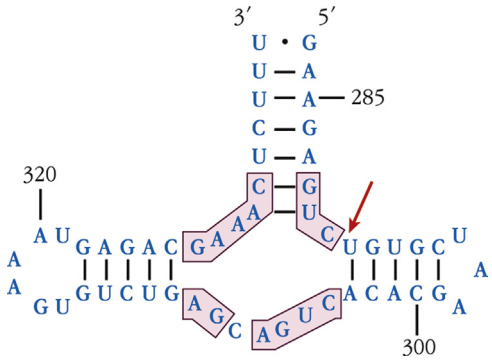


FIGURE 20.5 Predicted secondary structure of hammerhead ribozyme of plant viroids. The nucleotides conserved in all hammerhead ribozymes are boxed and shaded, the site of cleavage is marked by an arrow. Note that the ribozyme acts only once, because the segment harboring the cleavage site constitutes the catalytic unit of the ribozyme. The 5′ and 3′ termini of the ribozyme segment are denoted.

secondary structure of the viroid RNA promotes the ligation reaction to form a circle. Nonetheless, many fundamental questions regarding the viroids remain unresolved. For instance, how could viroid RNA infect plants without having a protein component? What is the underlying mechanism for the pathogenesis? What are the origins of viroids?

20.4 PRIONS AND MAD COW DISEASE

Prion[2] is an etiologic agent of transmissible neurodegenerative diseases. In contrast to viroids, prions are constituted of "protein only" and have no nucleic acid components. The prion disease best known to the public is the so-called mad cow disease. Here, the features of prion diseases are described with an emphasis on mad cow disease.

Discovery of Prions: Prion is a transmissible agent that was first isolated from a sheep afflicted by a disease termed "*scrapie*,"[3] which is a transmissible spongiform encephalopathy (TSE) of sheep. Scrapie was described as early as the 18th century in England, France, and Germany, but it only became a subject of scientific investigation after the 1990s. Scrapie was first known as an infectious disease in farm animals. The characterization of the scrapie agent as a "protein only" agent by Stanley Prusiner brought debates on the biological nature of the agent. He named it a "proteinaceous infectious agent" or "prion" to emphasize its lack of a nucleic acid component. The prion hypothesis was a "revolutionary thought," and it is still not widely accepted (Box 20.1).

Prions Diseases: Fatal neurodegenerative diseases similar to scrapie were found in human as well as in animals (Table 20.4). Histological examination of diseased brain tissues revealed the presence of vacuoles (ie, microscopic "holes" in the gray matter), which is seen as a sponge-like appearance (Fig. 20.6A). Reflecting the histological feature, these neurodegenerative diseases are collectively termed "TSE." Moreover, *amyloid*[4] fibrils were observed by electron microscopic examination of samples isolated from the tissues (Fig. 20.6B). It is speculated that amyloids are responsible for the pathological lesions found in TSE. On the other hand, all experimental evidence supports the lack of nucleic acids (DNA or RNA) in the agents, which leads to the "prion hypothesis."

Some pathological features are notable. First, spongiform encephalopathy, which is the loss of neuronal cells, represents the disease pathology. Amyloid plaque observed in the afflicted brain tissue is thought to be responsible for the cell death. Second, unlike most other infectious diseases, the lesions are not associated with inflammation. Third, the latency, a period from the primary infection to the onset of disease, is characteristically long (eg, many years). This pattern of infection is termed "slow infection," as exemplified in HIV infection (see Box 9.1). Fourth, the disease outcome is always fatal.

Importantly, prion diseases were found in human as well (see Table 20.4). The first human prion disease reported was "*Kuru*" that was described in the 1950s. Kuru was transmitted among members of the Fore tribe of Papua New Guinea via funerary cannibalism (Fig. 20.7). The etiological nature of the fatal disease endemic to tribal regions of

2. **Prion** The word *prion*, is derived from the words *protein* and *infection*, in reference to a prion's ability to self-propagate and transmit its conformation to other prions.

3. **Scrapie** It refers to a fatal, degenerative disease that affects the nervous systems of sheep and goats. The name "scrapie" is derived from one of the clinical signs of the condition, wherein affected animals will compulsively scrape off their fleeces against rocks, trees, or fences.

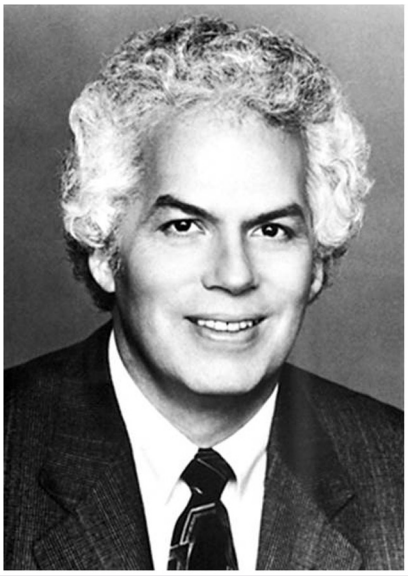
4. **Amyloid** It refers to the insoluble fibrous "protein aggregates" sharing specific structural traits.

BOX 20.1 Prion Hypothesis

A few outstanding features of prion disease including its transmissible nature and neurodegenerative disease manifestation made scientists interested in prion diseases. As a matter of fact, the debate on their etiological nature is still ongoing. Nobel Prizes were awarded to two visionary scientists, who set a keystone on our current knowledge of prion disease. First, Peter Gajdusek was awarded the Nobel Prize in 1976 for his work on Kuru. Kuru is an incurable degenerative neurological disorder endemic to tribal regions of Papua New Guinea. The etiology of Kuru was then enigmatic. Peter Gajdusek became interested in the disease and obtained brain tissues of Kuru victims from his colleague in Australia. He injected two chimpanzees with the materials isolated from brain tissue samples of Kuru victim. Within 2 years, one of the chimps had developed Kuru, demonstrating that Kuru is transmissible through inoculation of the agent and that the agent was capable of crossing the species barrier to other primates. This work defines the etiology of Kuru as a transmissible agent.

Another major advance in our understanding on prion disease was made by Stanley Prusiner. Based on early experimental evidence revealing the lack of nucleic acids in scrapie sample, he postulated that "proteineous infectious" materials are etiologic entities or the so-called the protein-only hypothesis.

In this work, he coined the term *prion*, which comes from the words "proteinaceous" and "infectious," in 1982 to refer to a previously undescribed form of infection due to protein misfolding. Stanley Prusiner received the Nobel Prize in Physiology or Medicine in 1997 for his work in proposing an explanation for the cause of BSE (mad cow disease) and its human equivalent, Creutzfeldt-Jakob disease.



The photos of two pioneers in prion research. (Left) Peter Gajdusek and (right) Stanley Prusiner.

Papua New Guinea was then enigmatic. It was Peter Gajdusek, who first demonstrated Kuru in chimpanzee by injecting infected materials, revealing the transmissible nature of the disease (see Box 20.1).

Although Kuru was the first human prion disease described, *Creutzfeldt-Jacob disease (CJD)* and *Gerstmann-Straussler syndrome (GSS)* are more prevalent prion diseases in human (see Table 20.4). CJD (sporadic CJD or sCJD) is the most common prion disease in human, nonetheless, only one in million per year is afflicted. CJD can be familial (ie, fCJD). Most cases of CJD (ie, 85%) are sporadic, while a fraction (ie, 10−15%) are familial. In the familial prion disease, a mutation occurs in the gene for PrP (ie, PRNP gene). GSS is much rarer (one in 10−100 million per year) and it is familial and associated with a certain mutation in the gene for PrP (ie, P102L mutation or proline to leucine substitution in amino acid number 102). In addition to these human prion diseases, a novel CJD was more recently reported, in which the disease pathology is somewhat distinct from CJD. It was later termed a *variant CJD* (vCJD).

Mad Cow Disease: Until the 1970s, scrapie was the representative prion disease in animals. Prion diseases in other animals, such as transmissible mink encephalopathy (TME) in minks and chronic wasting disease (CWD) in deer and elk, did not get much attention, because the cases were extremely rare. However, prion disease in cow, termed "mad cow disease," first reported in 1985 in the United Kingdom drew a great deal of public attention and became the

TABLE 20.4 The Important Features of Human and Animal Prion Diseases

Human Prion Diseases	Features (Cause, Epidemiology)	Symptoms
Creutzfeldt-Jacob disease (CJD)		
Sporadic CJD	Sporadic, aging	Dementia
Familial CJD	PrP mutation	Dementia
Variant CJD (vCJD)	Human mad cow disease	Progressive dementia
Gerstmann-Straussler syndrome (GSS)	PrP mutation	Fatal neurodegenerative disease
Kuru	Cannibalism (Papua New Guinea)	Fatal neurodegenerative disease
Fatal familial insomnia	PrP mutation	Insomnia
Animal prion diseases	**Host**	**Symptoms**
Scrapie	Sheep, goat	Fatal neurodegenerative disease
Bovine spongiform encephalopathy (BSE)	Cattle	Fatal neurodegenerative disease
Transmissible mink encephalopathy (TME)	Mink	Fatal neurodegenerative disease
Chronic wasting disease (CWD)	Deer and Elk	Fatal neurodegenerative disease

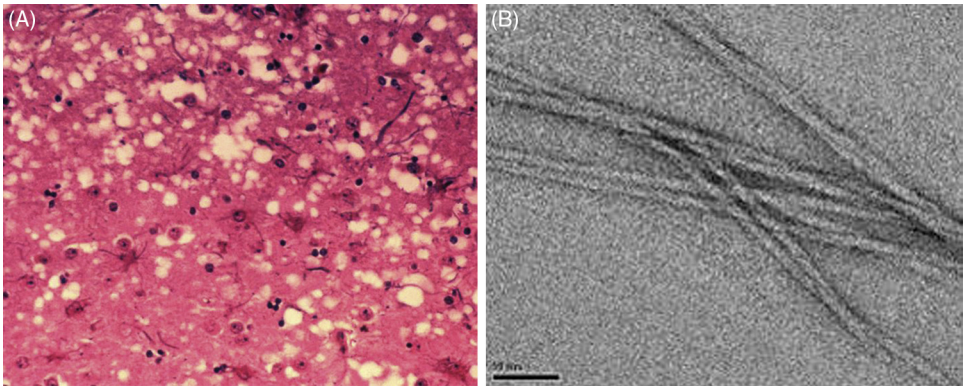


FIGURE 20.6 Pathological lesions in prion diseases and amyloid fibril. (A) This micrograph of brain tissue reveals the histologic lesions seen in BSE. The presence of vacuoles (ie, microscopic "holes" in the gray matter) gives the brain of BSE-affected cows a sponge-like appearance. (B) Electron micrograph image of the amyloid fibrils reconstituted in vitro by the prion peptide.

representative prion disease in animals. It is formally termed *bovine spongiform encephalopathy* (*BSE*).[5] In the United Kingdom, the country that was most affected, more than 180,000 cattle were infected and 4.4 million slaughtered during the eradication program.

In the past, the slaughtered meats of the afflicted animals were recycled as animal feeds after inactivation treatment (heat and chemical treatment) that would completely kill viruses, because scrapie was then regarded as a virus. The epidemic was believed to be caused by the recycling of slaughtered animals for animal feeds prior to recognition of etiological nature of prion diseases. Since 1988, ruminant protein feed was banned to stop the epidemic. Nevertheless, a new form of neurodegenerative disease, which is similar to sporadic CJD, began to occur in 1992 (Fig. 20.8); it was termed as a vCJD But it is commonly known as "human mad cow disease." By June 2014 it had killed 177 people in

5. **Bovine spongiform encephalopathy (BSE)** It is a fatal neurodegenerative disease (encephalopathy) in cattle that causes a spongy degeneration in brain and spinal cord.

FIGURE 20.7 A group of Kuru patients in 1957 at New Guinea. Kuru patients can only stand with the aid of the sticks. All died within 1 year of the photograph being taken. The term "kuru" derives from the Fore word "kuria/guria" (to shake), a reference to the body tremors that are a classic symptom of the disease.

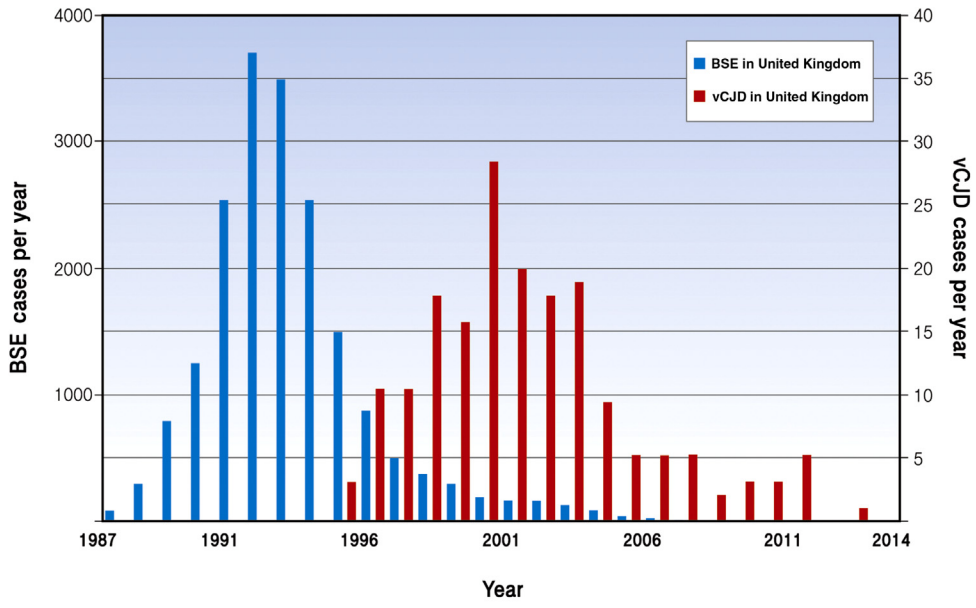


FIGURE 20.8 The relationship between BSE and vCJD. The epidemiologic relationship between BSE and vCJD is evident, as the bell shaped curve of BSE cases is shadowed by the vCJD cases with a gap of about 20 years in between. The gap might represent the latent period for the onset of disease after primary infection.

the United Kingdom, and 52 elsewhere. A causal link between vCJD and BSE was speculated in the early 1990s. The controversy over the cause of vCJD was concluded by the UK government's acknowledgment on the causal relationship between BSE and vCJD in 1996. Thanks to the implementation of stringent monitoring of slaughtered meat products, vCJD as well as "mad cow disease" is practically eradicated.

Prion Hypothesis: The "prion hypothesis" or "a protein-only hypothesis" states that a protein is the only etiologic component of the pathogen that causes the disease. What is the evidence for the lack of nucleic acids in prion agents? Four kinds of biochemical evidences were obtained. First, the infectivity of prions is not inactivated by heating to 90°C for 30 min (or even at 360°C for 1 h), a condition that would completely inactivate any nucleic acid. Second, the infectivity of scrapie agents is resistant to the inactivation by UV radiation and ionizing radiation. Because UV radiation and

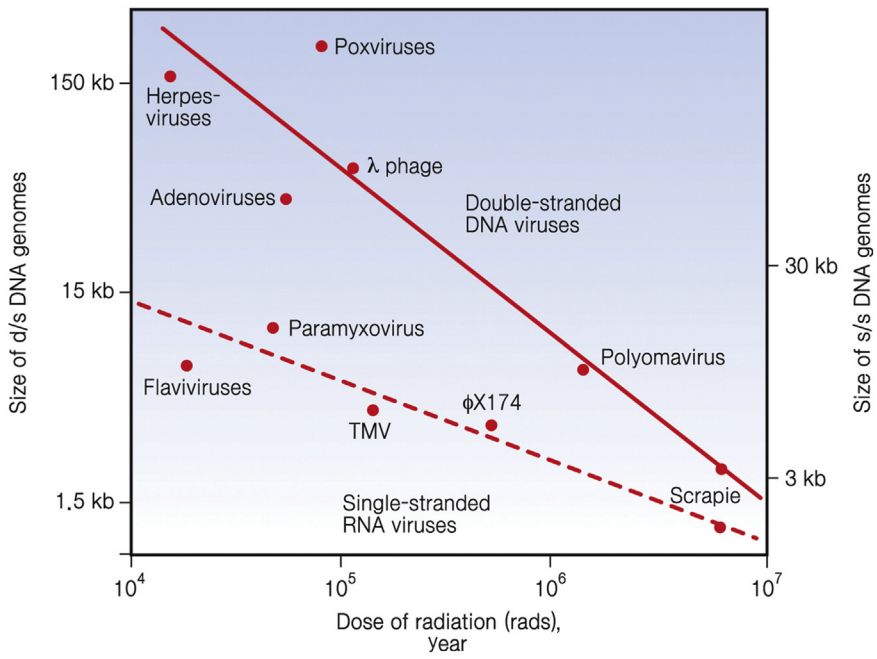


FIGURE 20.9 Correlation between the virus genome size and susceptibility to UV radiation. Inactivation radiation doses of various DNA (solid line) and RNA viruses (dotted line) are plotted. Larger genomes present a larger target and therefore are more sensitive to the inactivation of infectivity by UV than smaller genome. The estimated genome size of the scrapie agent is less than 1 kb, if any. Note log scale on vertical axis.

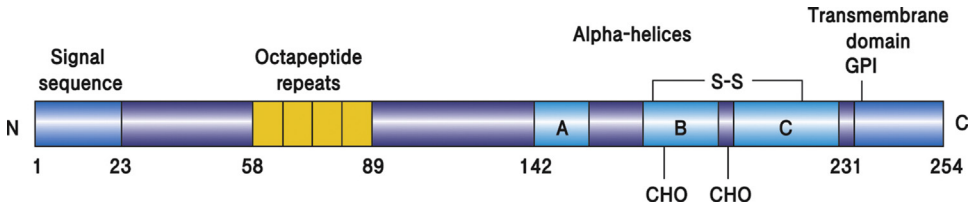


FIGURE 20.10 Protein domains of PrP. The N-terminal signal sequence targets it to the surface of cells. Three α-helical structures (A, B, and C) are denoted. The glycosylation sites are denoted by CHO. A glycosylphosphatidylinositol (GPI) membrane anchor at the COOH-terminal tethers PrP to cell membranes.

ionizing radiation inactivates infectious organisms by causing damage to their nucleic acid genome, there is an inverse relationship between the size of nucleic acid genome and the dose of UV radiation needed for the inactivation (Fig. 20.9). The scrapie agent was found to be highly resistant to both UV light and ionizing radiation, indicating that any nucleic acid present must be extremely small, probably less than 1 kb nucleotides. Third, scrapie agent was resistant to DNase and RNase treatment but sensitive to proteinase treatment, indicating that the infectivity is conferred by proteins. Fourth, scrapie was sensitive to any protein-denaturing agents such as urea, sodium dodecyl sulfate, phenol, and other chaotropic agents.

What is, then, the identity of the protein components of prion agents? Stanley Prusiner found a novel protein with a size of 27−30 kDa in a fraction with higher infectivity. It was termed *PrP* (prion protein) (Fig. 20.10). Surprisingly, the gene for this prion-associated protein is coded by the host chromosome. It was totally unexpected that a gene for the infectious agent is a host gene. In retrospect, the lack of inflammation in prion diseases is consistent with the causative role of host encoded protein. No immune response is invoked, because the infectious agents are self rather than nonself.

It was noted that PrP in scrapie animals is distinct from the one in normal animals in some biochemical properties such as protease-sensitivity. It turned out that the secondary structure of PrP in scrapie animals is distinct from that in normal animals (Fig. 20.11). The PrP in a normal cell has a largely α-helical structure, whereas the PrP in scrapie animals are abundant in a β-sheet structure. Importantly, the conformation change of PrP protein is associated with the disease. The endogenous and properly folded form is denoted PrP^{C} (for *Cellular*), whereas the disease-linked and misfolded form is denoted PrP^{Sc} (for *Scrapie*). PrP^{Sc} forms amyloid fibrils, leading to amyloid plaque formation. The cell death induced by amyloid plaque, eventually results in spongiform encephalitis. Prion disease is often referred to as "protein folding disease," emphasizing the unprecedented etiology involving the conformation change of the disease protein.

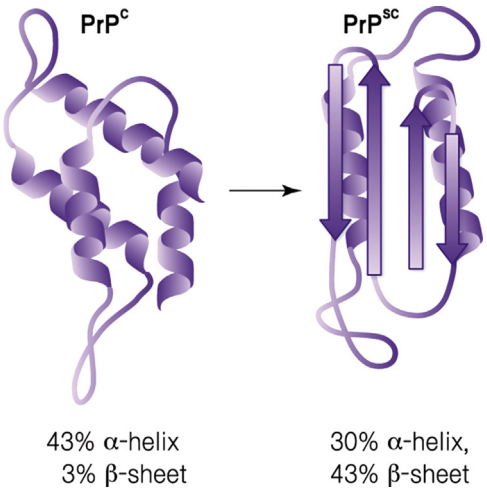


FIGURE 20.11 The protein structure of PrP^{C} and PrP^{SC}. The proportion of α-helical and β-sheet structures are denoted below the protein structures of PrP^{C} and PrP^{SC}. PrP^{SC}, which has a significant portion of β-sheet structures, represents a pathogenic conformer of PrP isoform, and it is an aggregation-prone isoform of PrP^{C}.

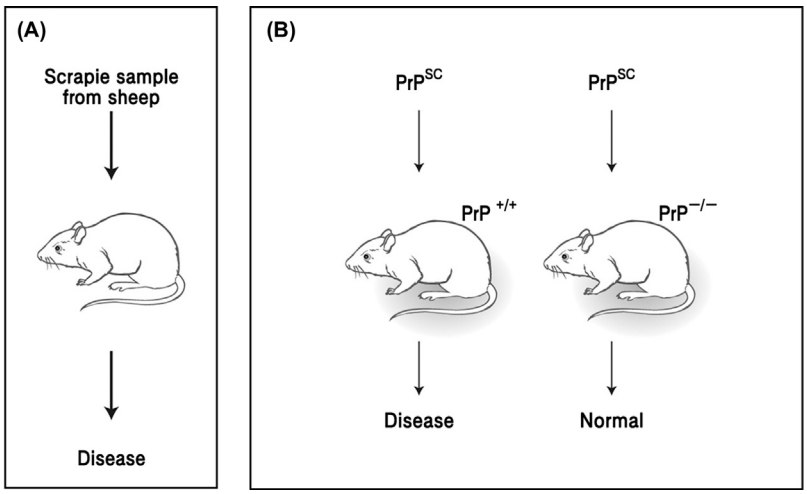


FIGURE 20.12 Animal model for prion diseases. (A) Establishment of a mouse model to study prion diseases. Intracerebral injection of scrapie agents into a mouse leads to the diseases. PrP^{SC} formation was confirmed by trypsin-resistance. A transgenic mouse that overexpresses PrP^{C} protein facilitates the disease manifestation with a shorter latent period. (B) Intracerebral injection of PrP^{SC} sample to a wild-type mouse leads to the prion disease, whereas intracerebral injection of PrP^{SC} sample to a PrP^{C}-knockout mouse did not lead to the prion disease.

To prove a protein-only hypothesis, one needs to demonstrate the infectivity of PrP in in vivo circumstances. An animal model for the prion disease has been established in mouse by intracerebral injection of scrapie agents isolated from a scrapie infected sheep (Fig. 20.12A). Remarkably, pathological lesions including spongiform encephalopathy were observed in the brain of mouse about 3 months after the intracerebral administration. Recapitulation of prion diseases in the mouse model enabled the investigation of the pathogenesis of prion disease in a laboratory. An experiment performed using PrP gene knockout mouse further corroborated the prion hypothesis (Fig. 20.12B). An intracerebral injection of PrP^{Sc} sample to a PrP knockout mouse did not cause the disease, revealing that a PrP knockout mouse is resistant to the disease. Moreover, this finding supports the notion that the protein−protein interaction between PrP^{C} and PrP^{SC} is critical for the disease manifestation.

After all, the prion hypothesis was largely substantiated by the finding described above. In summary, (1) an etiological agent of prion disease is a structural isoform (ie, PrP^{Sc}) of a host-coded PrP^{C} protein, (2) PrP^{Sc} is essential for the conversion of PrP^{C} to PrP^{Sc}, and (3) PrP^{C} exists in monomer, while PrP^{Sc} tends to aggregate to form a multimeric form. A question is, then, how can the amount of infectious agent (ie, PrP^{Sc}) be amplified? The amplification of PrP^{Sc} can be attained by the conversion of PrP^{C} to PrP^{Sc} via a protein−protein interaction in that PrP^{Sc} is a template for the conversion of PrP^{C} to PrP^{Sc} (Fig. 20.13). In doing so, the amount of PrP^{Sc} increases.

The fact that a Nobel Prize was awarded to Stanley Prusiner did not necessarily vindicate the protein-only hypothesis. Others still insisted that PrP^{Sc} represents only a subset of infectious materials, arguing the presence of other

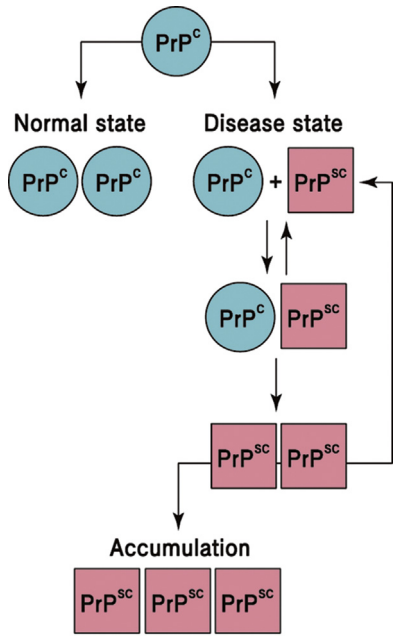


FIGURE 20.13 A model that accounts for the propagation of prion agents. A model states that the conformation change induced by the protein–protein interaction between PrP^{C} and PrP^{SC} underlies the propagation of prion agents. Specifically, PrP^{C} is converted into PrP^{SC} upon an interaction with PrP^{SC} monomer, forming a dimer or multimer. Accumulation of PrP^{SC} leads to the formation of amyloid fibrils. Breakage of PrP^{SC} nucleus recycles to amplify PrP^{SC}.

cofactors. The final proof would be a successful demonstration of the disease pathology in the mouse model following intracerebral injection of highly purified recombinant PrP^{Sc} or synthetic PrP^{Sc} made in vitro. Recently, recombinant PrP^{Sc} was successfully generated in vitro from bacterially expressed recombinant PrP^{Sc} protein (see Journal Club). This seminal work proved that the infectivity of the prion disease results from an altered conformation of PrP^{C}. Although the molecular attributes of prion diseases became clear, the pathogenesis of prion disease remains uncertain. It still remains unclear how prions damage neuronal cells.

20.5 PERSPECTIVES

Two subviral agents, including viroids and prions, deserve a great deal of attention, because they appear to represent the most simplistic infectious agents that one could imagine. They are distinct from other conventional infectious agents with respect to their biochemical constituents, replication mechanism, and host interaction. Viroids are "RNA only" agents, while prions are "protein-only" agents. Regarding viroids, many fundamental questions have not been answered. For instance, it remains uncertain whether cellular RNA polymerase II is entirely responsible for RNA synthesis, and if so, how cellular RNA polymerase II, which normally does not utilize RNA as a template, can utilize viroid RNA as a template. Another important question is how the RNA itself can transmit, and how RNA can induce pathologic lesions in plants. Regarding prions, a great advance has been made in our understanding on the peculiar nature of the disease etiology. Nonetheless, some salient questions remain unanswered. For instance, it is unclear how prions enter the body and reach the central nervous systems (CNS). This raises an intriguing question of how a mere protein aggregate can trespass mucosal barriers, circumvent innate and adaptive immunity, and travel across the *blood–brain barrier*[6] to eventually cause brain disease. Another unanswered question is what is the physiological function of PrP^{C} besides serving as a substrate for the generation of PrP^{Sc}? Lastly, how does PrP^{C} misfolding cause neurological disease? These intriguing questions provide challenges for young ambitious scientists.

6. **Blood–brain barrier** It refers to a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the CNS. It allows the passage of water, some gases, and lipid soluble molecules by passive diffusion, as well as selective transport of molecules such as glucose and amino acids that are crucial to neural function.

20.6 SUMMARY

- *Subviral agents*: Some transmissible agents are classified as subviral agents as they do not fit into the conventional definition of "virus." These include satellite viruses, viroids, and prions.
- *Satellite virus*: Satellite virus depends on another virus, a host or helper virus, for propagation. Adeno-associated virus (AAV) is a satellite virus of adenovirus.
- *Viroids*: Viroids are infectious agents found in plants that constitute of only a small circular RNA molecule without a protein coat. The ribozyme was discovered in viroid RNA.
- *Prions*: Prions are transmissible agents that are constituted of "protein only," and are devoid of nucleic acid components. Conversion of cellular prion protein (PrP^{C}) to a pathogenic isoform (PrP^{Sc}) via protein−protein interaction is the underlying mechanism for prion diseases. The best-known example of prion disease for the general public is so-called mad cow disease in cattle.

STUDY QUESTIONS

20.1 Satellite viruses cannot replicate in the absence of a helper virus. Answer the following question on satellite viruses. (1) Relatedness to host viruses. (2) Particle morphology, and (3) Impact on the growth of helper virus.

20.2 Rolling-circle mechanism has been proposed to account for the viroid RNA replication. (1) List three enzymatic activities that are required to account for the rolling-circle mechanism. (2) Explain how viroids attain these enzymatic activities despite the lack of coding capacity.

20.3 State three experimental evidences that are in favor of a protein-only hypothesis.

SUGGESTED READING

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JOURNAL CLUB

- Wang, F., Wang, X., Yuan, C.G., Ma, J., 2010. Generating a prion with bacterially expressed recombinant prion protein. Science 327, 1132−1135.

  Highlight: This seminal paper described the generation of a highly infectious prion in vitro by using PrP purified from *Escherichia coli*. A recombinant prion has the attributes of the pathogenic PrP isoform: aggregated, protease-resistance, and self-perpetuating. After intracerebral injection of the recombinant prion, remarkably, wild-type mice developed neurological diseases in 150 days and succumbed to prion disease. This work proved that the infectivity in prion disease results from an altered conformation of PrP.