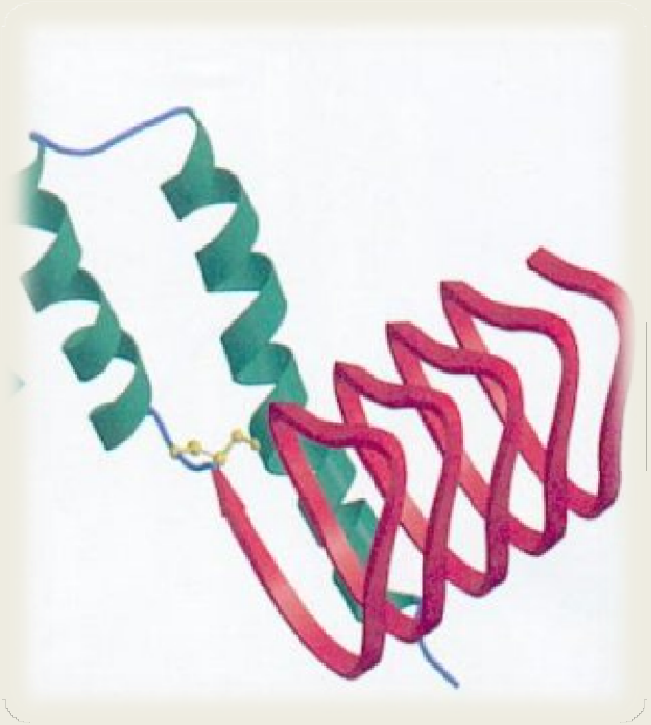
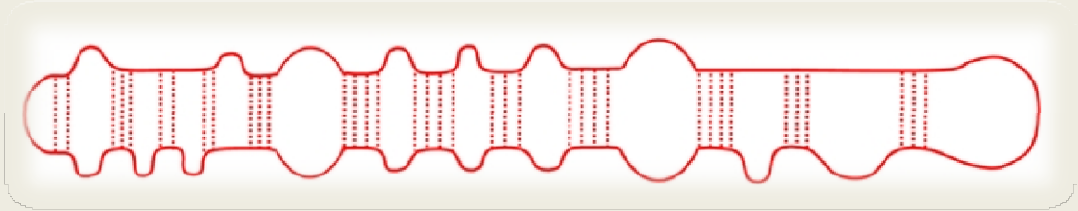
Sub viral particles





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Sub viral particles

- Viruses are not the only submicroscopic entities capable of causing disorders within cells.

- Sub viral particles are infectious agents that resemble viruses in structure and composition. But they are notably smaller and simpler than viruses but which lack either nucleic acid or protein and are thus not viruses.
- (Thus they may be proteins without nucleic acids or only nucleic acids without protein coat /capsid.)

- There are 3 sub viral agents.

1. Prions

2. Viroids

3. Virusoids

1. Prions

History and discovery

- In 1982, Stanley Prusiner described a proteinaceous infectious agent that was different from any other known infectious agent in that **it lacked instructional nucleic acid**.

- Prusiner named such agents of disease prions, for *pr*oteinaceous *in*fective particles.

- Before his discovery, the diseases now known to be caused by prions were thought to be caused by what were known as "slow viruses", which were so named because 60 years might lapse between infection and the onset of signs and symptoms.

- Later, the major protein constituent of the infectious agent was sequenced and named the prion protein (PrP), to reflect the possibility that it was the sole necessary constituent of the infectious agent, designated the prion.

- Prusiner and his colleagues showed that prions are not viruses because they lack any nucleic acid. **Stanley Prusiner** awarded the Nobel prize in Medicine in 1997.

- Prions are infectious sub viral particles of **misfolded proteins**, **devoid of nucleic acids.** They are extremely simple and smaller than the smallest virus.

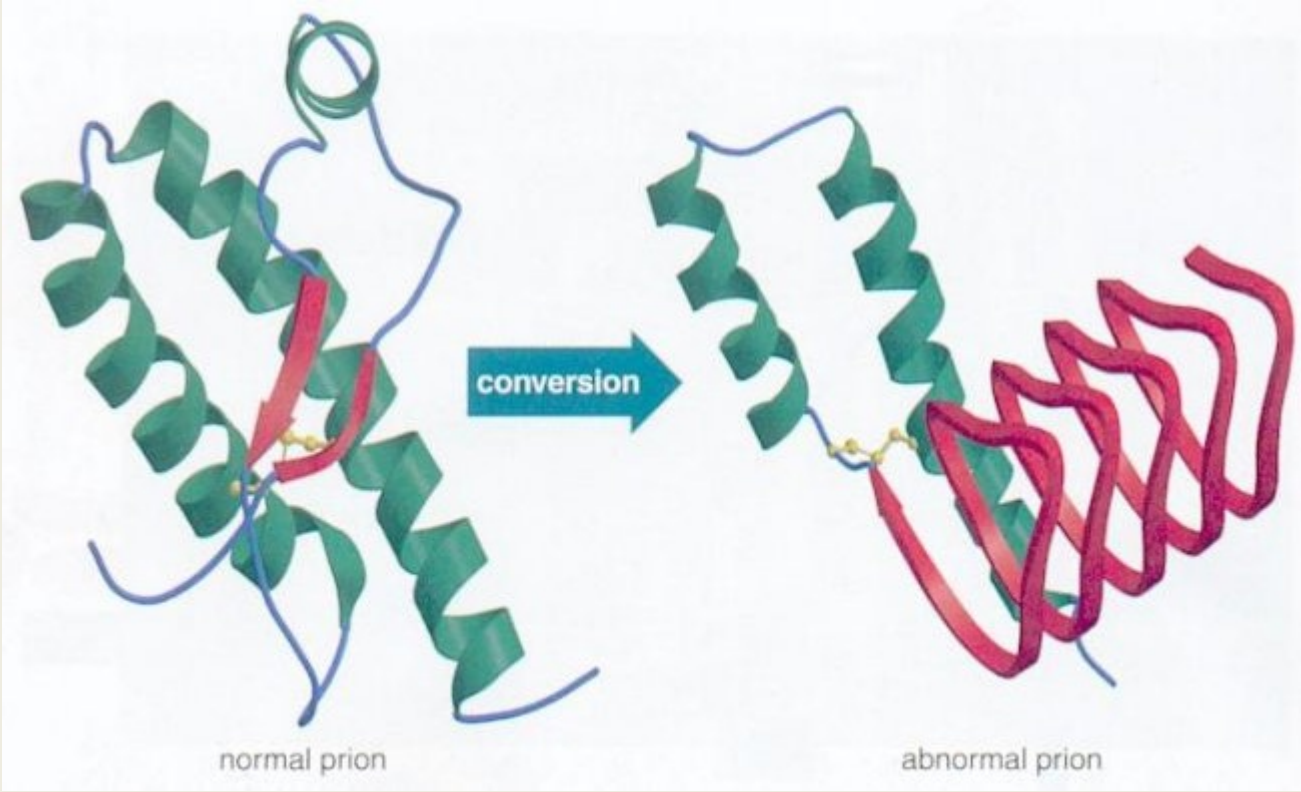
- Since prions are devoid of nucleic acid ,they are not self replicating.
- They have the powers to resist inactivation by procedures which destroy nucleic acids.
- The abnormal folding of prions is due to a defect in the gene that codes for the prion protein (PrP).
- The PrP coded by the defective gene , has proline ,instead of **leucine.** This probably causes the abnormal folding of the protein.

- They are believed to produce new prions by *protein directed protein* production.
- This is a deviation from the normal DNA directed protein synthesis. The exact molecular mechanism is not clearly understood.
- Large number of abnormal prion proteins clump together in the brain and cause neurological diseases.
- Structurally prions are rod shaped and mainly found on the surface of brain cells and some other cells.
- Each rod is formed as many as 1000 molecules of PrP.

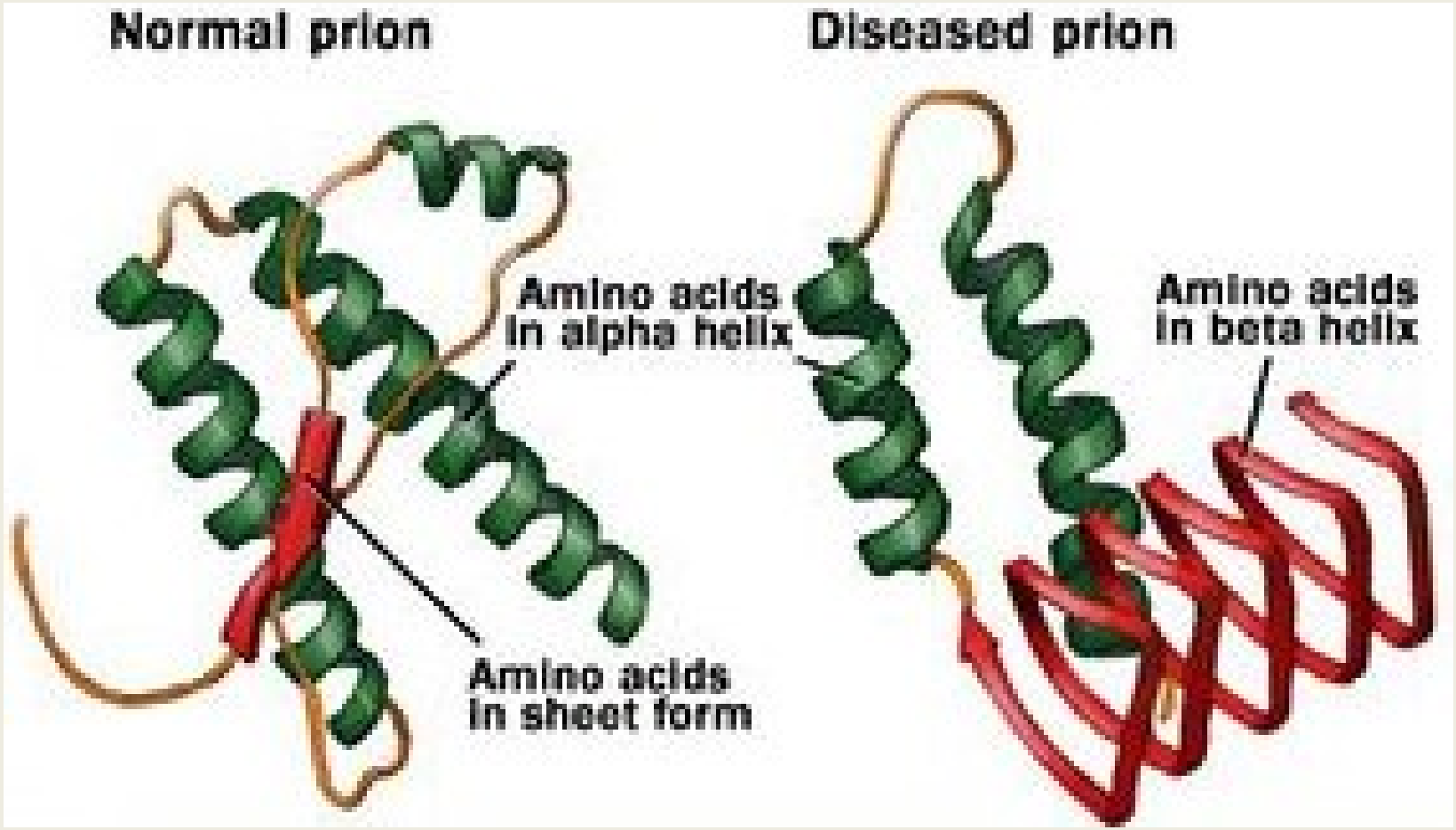
- Even then they can infect host cells , initiate the production of new prions and can cause transmissible neurological (neurodegenerative) diseases.

Sub viral particles - Prions

- PrP is a host-encoded protein which exists as PrP^{C} (cellular) in the non-infected host, and as PrP^{Sc} (scrapie) as the major component of the scrapie infectious agent.



PrP^{C} PrP^{Sc}


Normal prion
Diseased prion
Amino acids in alpha helix
Amino acids in sheet form
Amino acids in beta helix

Prion diseases

- Prion disease is the new designation of a group of spongiform encephalopathies, all invariably fatal, which show similar clinical and neuropathological changes.

- They comprise a range of distinct diseases in both animals and man, and spontaneous, hereditary and transmissible forms are recognized.

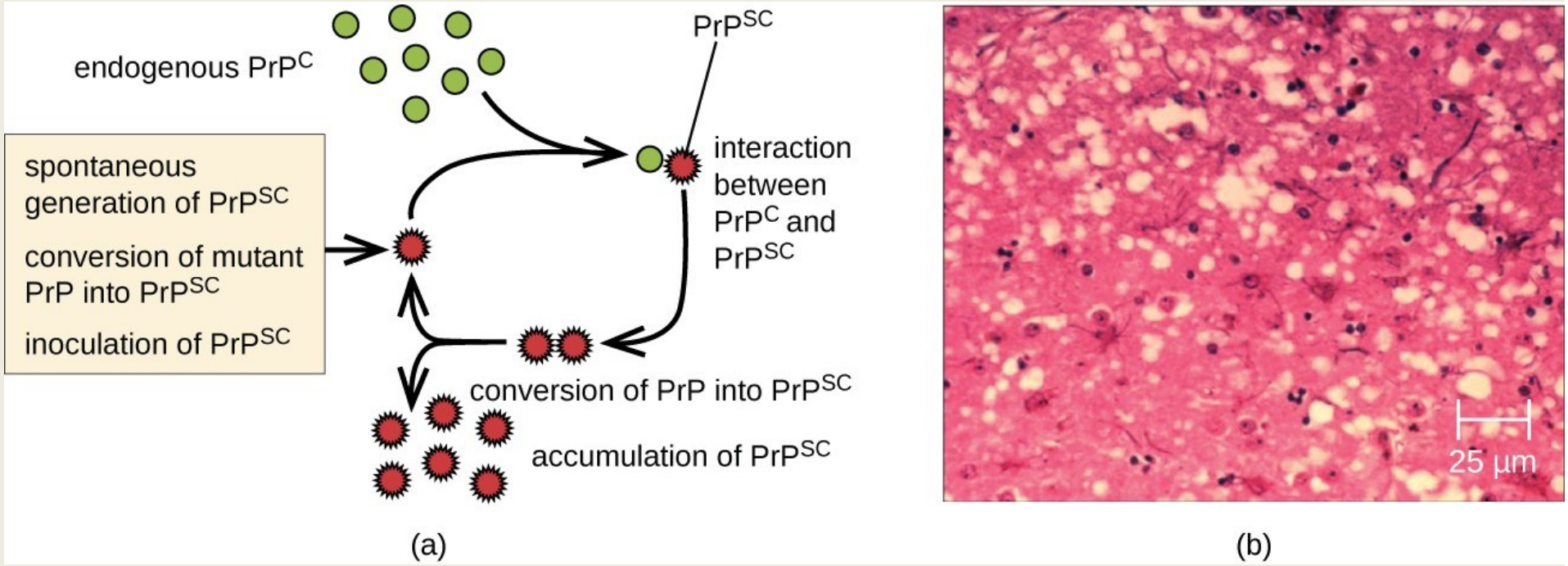
- Prion diseases are transmissible, progressive and invariably fatal neurodegenerative conditions associated with misfolding and aggregation of a host-encoded cellular prion protein, PrP(C).

- They have occurred in a wide range of mammalian species including human that manifest primarily as progressive dementia and ataxia.

- Unique to these diseases is the prion, that can transmit disease from cell to cell or host to host by associating with, and transforming, normal prion protein into the misfolded isoform (the pathogenic scrapie-inducing form).

- Prions are the causative agents of some chronic and transmissible neurodegenerative disease in sheep, cattle, deer etc.

- Humans are also susceptible to several prion diseases .


endogenous PrP^C
PrP^SC
spontaneous generation of PrP^SC
conversion of mutant PrP into PrP^SC
inoculation of PrP^SC
interaction between PrP^C and PrP^SC
conversion of PrP into PrP^SC
accumulation of PrP^SC
25 µm
(a)
(b)

Prion diseases in animals

- **Prions (for *proteinaceous infectious particle)*** cause a variety of neurodegenerative diseases in humans and animals. **Prion diseases** are often called spongiform encephalopathies.

1. Scrapie disease in sheep

- The best studied prion is **the scrapie prion**, which causes the disease scrapie in sheep.

- Afflicted animals lose coordination of their movements, tend to scrape or rub their skin, and eventually cannot walk. Scrapie is a fatal, degenerative **disease** that affects the nervous systems of sheep and goats.

- Researchers have shown that scrapie is caused by an abnormal form of a cellular protein.

- The abnormal form is called **PrPSc** (for *scrapie-associated prion protein), and the normal cellular form is* called **PrPC**.

- Evidence supports a model in which entry of PrPSc into the brain of an animal causes the PrPC protein to change from its normal conformation to the abnormal form.

- The newly produced PrPSc molecules then convert more PrPC molecules into the abnormal PrPSc form.

- How the PrPSc causes this conformational change is unclear.

- However, the best-supported model is that the PrPSc directly interacts with PrPC, causing the change.

2. Bovine Spongiform Encephalopathy (BSE or "mad cow disease")

- In addition to scrapie, prions are responsible for **Bovine Spongiform Encephalopathy** (BSE or "mad cow disease"), and few human diseases.

- Mad cow disease reached epidemic proportions in Great Britain in the 1990s and initially spread because cattle were fed meal made from all parts of cattle including brain tissue.

- It has now been shown that eating meat from cattle with BSE can cause a variant of Creutzfeldt-Jakob disease in humans (vCJD).

Prion diseases in Humans

- Prion diseases are a group of diseases caused by abnormally conformed infectious proteins, called prions.

Four major neurological human disease are known to be caused by prions.

1. Creutzfeldt-Jakob Disease (CJD) and vCJD - sporadic CJD is the most common form of human prion disease.

2. Familial Fatal Insomnia (FFI) – genetic in origin.

(Genetic prion diseases are caused by mutations in the prion-related protein gene (PRNP))

It is a rare prion disease that interferes with sleep and leads to deterioration of mental function and loss of coordination. Death occurs within a few months to a few years.

3. Kuru- acquired in origin.

(Acquired prion diseases fortunately are becoming rarer, as awareness of transmission risk has led to implementation of measures to prevent such occurrences).

- The first human example of spongiform encephalopathy arose from the discovery of the infectious etiology of kuru.

- CJD is rare and cosmopolitan in distribution among middle-aged people, while **kuru** has been found only in the Fore, an eastern indigenous tribes of Papua New Guinea.

- This tribe had a custom of consuming dead kinsmen. Women and children were given the less desirable body parts to eat; this included the brain. Thus kuru was spread by ritual cannibalism.

- Thus they and their children were infected. Cannibalism was stopped many years ago, and kuru has been eliminated.

4. Alpers Syndrome - genetic in origin

- Alpers disease is a progressive neurologic disorder that begins during childhood and is complicated in many instances by serious liver disease. Symptoms include increased muscle tone with exaggerated reflexes (spasticity), seizures, and loss of cognitive ability (dementia).

- (A **seizure** is a sudden, uncontrolled electrical disturbance in the brain. It can cause changes in your behavior, movements or feelings, and in levels of consciousness. If you have two or more **seizures** or a tendency to have recurrent **seizures**, you have epilepsy.)

- Effects of prion diseases are fatal and devastating. All result in progressive degeneration of the brain and eventual death.

- They result either from the ingestion of the prion protein infected animals or from the mutation of the normal prion protein genes.

- At present, the mechanism of prions is imperfectly understood and so there is no effective treatment and have no cure.

- It has now been shown that eating meat from cattle with BSE can cause a variant of Creutzfeldt-Jakob disease in humans (vCJD).

- Acquired prion diseases in human can also be spread by corneal transplants with infected tissue or by contaminated cadaver-derived human growth hormone.

- More than 90 people have died in the United Kingdom and France from this source.

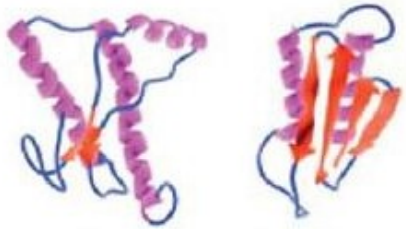
- Variant CJD differs from CJD in origin only:

	Creutzfeldt-Jakob Disease (CJD)	Variant CJD
Origin of disease	CJD is an extremely rare condition caused by **spontaneous mutation** of the gene that encodes the prion protein.	People acquire vCJD by eating **contaminated meat**

How Creutzfeldt-Jakob disease works

CAUSE

Creutzfeldt-Jakob disease is caused by abnormal proteins called prions that are not killed by standard methods for sterilizing surgical equipment.



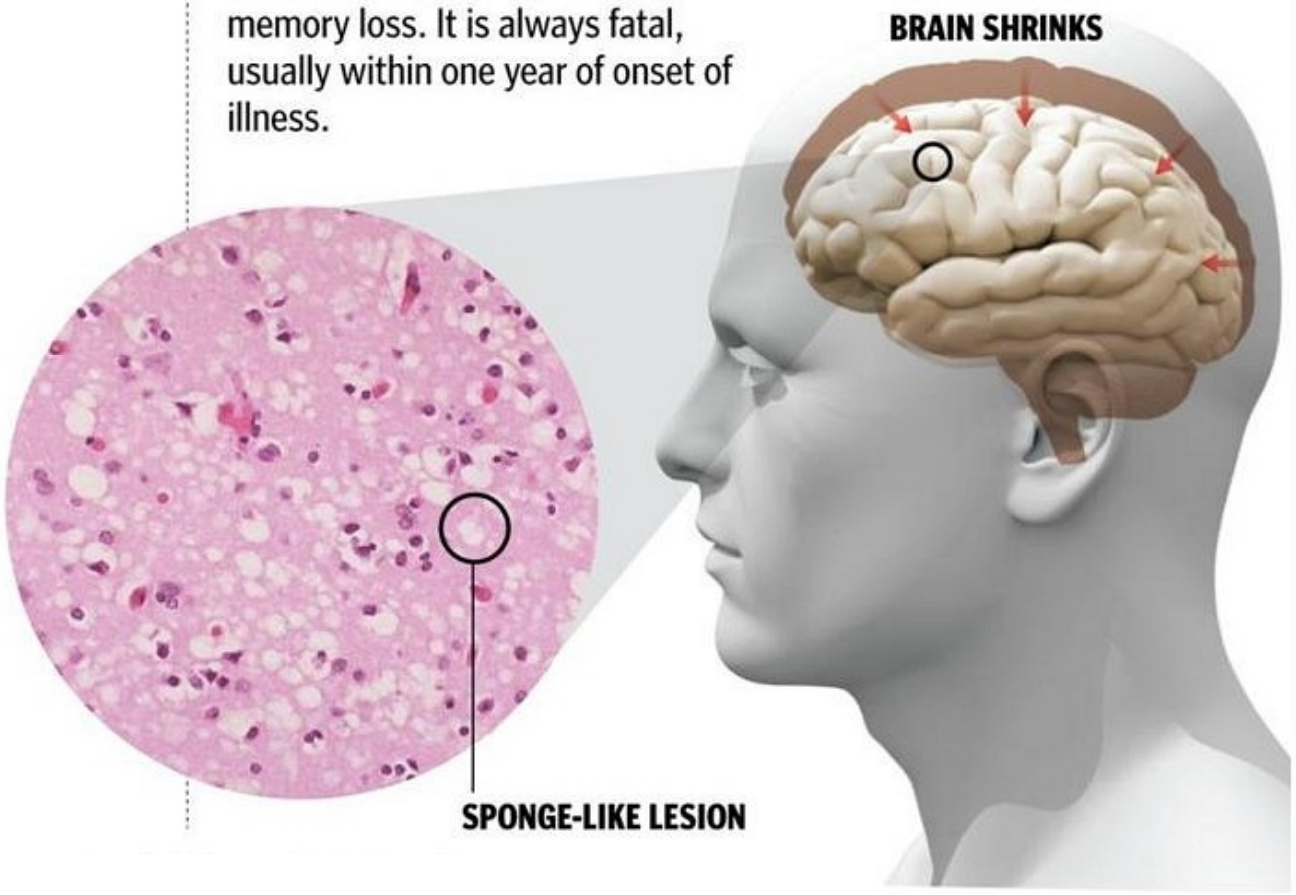
NORMAL HUMAN PROTEIN

DISEASE-CAUSING PRION

As prions build up in cells, the brain slowly shrinks and the tissue fills with holes until it resembles a sponge.

CONSEQUENCES

Those affected lose the ability to think and to move properly and suffer from memory loss. It is always fatal, usually within one year of onset of illness.

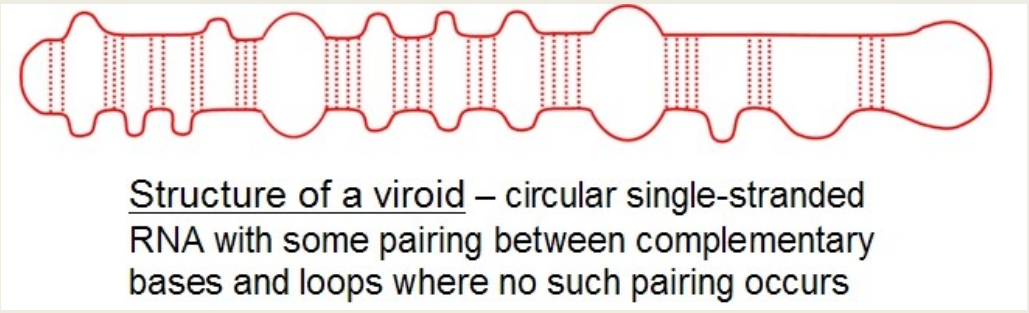


2. Viroids

- **Viroids are the smallest infectious pathogens known**, larger only than prions (nucleic acid-free, misfolded protein molecules, only whose abnormal conformation is transmitted, are not strictly infectious pathogens).

- Viroids consist solely of short strands of **circular, covalently closed, single-stranded RNA without protein coats.** They are mostly plant pathogens.

- **The circular RNA normally exists as a rodlike shape** due to intra strand base pairing, which forms double-stranded regions (stem) with single-stranded loops.



Structure of a viroid – circular single-stranded RNA with some pairing between complementary bases and loops where no such pairing occurs

- Viroids were discovered in 1971 by T.O. Diener, a plant pathologist.

- It is still not clear how they cause disease, especially since they may cause severe problems in one plant and no particular symptoms in a related species.

- They **encode no proteins**.

- Perhaps they bind to something in the cell and disrupt some regulatory mechanism.

- The RNA of many viroids contains sections of nucleotide sequence complementary to key regions at the boundaries of RNA introns; maybe that is how they damage cells.

- Viroid genomes are extremely small in size, ranging from 246 to 467 nucleobases. In comparison, the genome of the smallest known viruses capable of causing an infection by themselves are around 2,000 nucleobases in size.

- **Viroids cause over 20 different plant diseases,** including

1. Potato spindle-tuber disease
2. Exocortis disease of citrus trees
3. Chrysanthemum stunt disease.

- Some viroids are found in the nucleolus of infected host cells, where between 200 and 10,000 copies may be present.
- Others are located within chloroplasts.

- Potato spindle-tuber disease

Exocortis disease of citrus trees

- Symptoms of **bark scaling** and **severe stunting** usually developing when trees are around 4 years of age. When bark scaling occurs it appears as **cracking and peeling of the bark below the bud union**

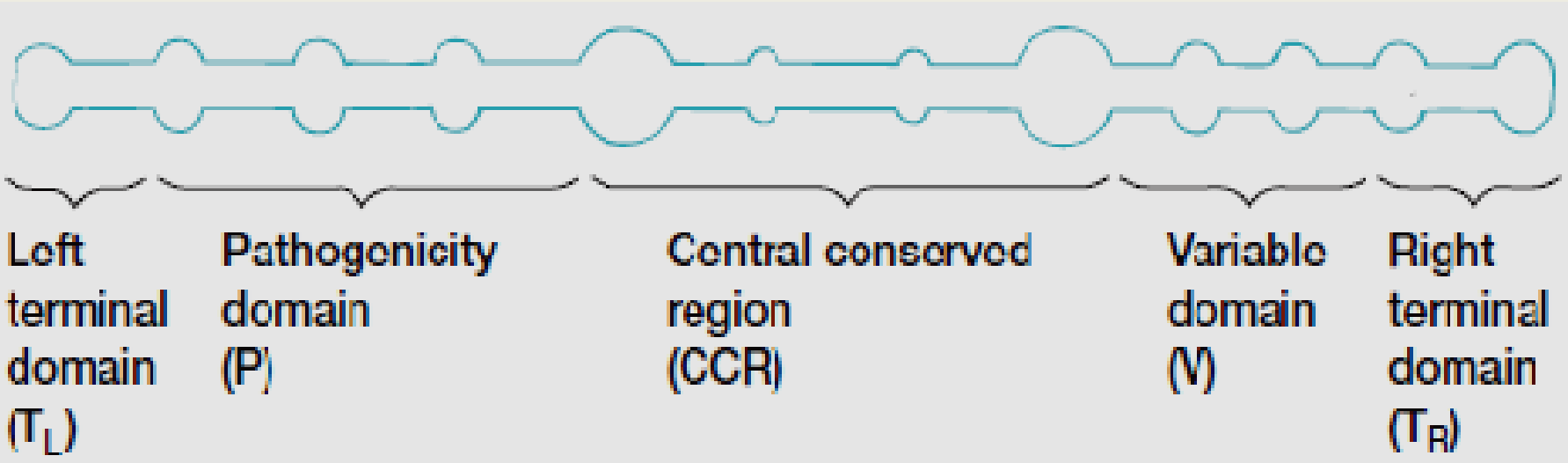


Figure 18.20 Viroid Structure. This schematic diagram shows the general organization of a viroid. The closed single-stranded RNA circle has extensive intrastrand base pairing and interspersed unpaired loops. Viroids have five domains. Most changes in viroid pathogenicity seem to arise from variations in the P and T_L domains.

- Interestingly, the RNA of viroids does not encode any gene products, so they cannot replicate themselves.

- Rather, it is thought that the viroid is replicated by one of the host cell's RNA-dependent RNA polymerases.

- **The host polymerase** evidently uses the viroid RNA as a template for RNA synthesis, rather than host DNA.

- The host polymerase synthesizes a complementary RNA molecule, a negative-strand RNA.

- This then serves as the template for the same host polymerase, and new viroid RNAs are synthesized. Both steps may occur by a rolling-circle- like mechanism.

- A plant may be infected with a viroid without showing symptoms—that is, it may have a latent infection.

- However, the same viroid in another host species may cause severe disease.

- All variations in pathogenicity are due to a few nucleotide changes in two short regions on the viroid. It is believed that these sequence changes alter the shape of the rod and thus its ability to cause disease.

- Since then **viroids have been found to differ from viruses** in six ways:

1. **Each viroid consists of a single circular RNA molecule** of low molecular weight, 246 to 399 nucleotides in length.

2. **Viroids exist inside cells, usually inside of nucleoli,** as particles of RNA without capsids or envelopes.

3. **Unlike viruses such as the parvoviruses, viroids do** not require a helper virus.

4. **Viroid RNA does not produce proteins.**

5. **Unlike virus RNA, which may be copied in the host** cell's cytoplasm or nucleus, viroid RNA is always copied in the host cell nucleus.

6. **Viroid particles are not apparent in infected tissues** without the use of special techniques to identify nucleotide sequences in the RNA.

Satellites

- Satellites are small, single-stranded RNA molecules, usually 500 to 2,000 nucleotides in length, that lack genes required for their replication.

- However, in the presence of a helper virus, they can replicate.

- There are two types:

1. Satellite viruses
2. Satellite nucleic acids (also known as virusoids).

- **They are called satellites because their reproduction** "revolves around" a helper virus.

- The satellite is defective in being unable to replicate alone.

- Satellite viruses may infect plants, animals, or bacteria. It encodes a capsid protein but an RNA polymerase encoded by the helper virus helps to replicates genome of satellite virus and its own.

- Satellite viruses does, however, have genes coding for the capsid that covers it, in contrast to satellite nucleic acids (virusoids), which are covered by a capsid coded for by their helper virus.

3. Virusoid

- Virusoids are circular single stranded RNAs dependent on a helper/ master virus for replication, encapsidation and to gain entry into a target cell.

- **Virusoids are similar to viroids in that they are also covalently** closed, circular, ssRNA molecules with regions capable of intra strand base pairing.

- The size of these RNAs is several hundred nucleotides long and does not code for any protein for capsid and replication.

- In contrast to viroids, virusoids typically need a helper virus in order to infect host cells. A virusoid genome does not code any proteins for their capsid and replication, but instead serves only to replicate itself.

- They live within the capsid core of another virus called the **helper virus.** They totally depend on the helper virus for their replication.

- This helper virus also encapsidated them.

- The helper virus supplies gene products and other materials needed by the virusoid for completion of its replication cycle.

- Virusoids can replicate in cytoplasm and possess a ribozyme activity.

Examples

1. Subterranean clover mottle satellite RNA (virusoid) : helper virus is sobemovirus.

2. The best-studied virusoid is the human hepatitis D virusoid, which is 1,700 nucleotides long.

- It uses the **hepatitis B virus** as its **helper virus.**

- If a host cell contains both the hepatitis B virus and the hepatitis D virusoid, the virusoid RNA and its gene product, called delta antigen, can be packaged within the envelope of the virus.

- These enveloped virusoids and delta antigens are capable of entering other host cells, where the virusoid RNA is transcribed by the host's RNA polymerase II.

Viral oncogenesis (Brief)

- A cancer is a tumor; it is an abnormal growth or enlargement of tissue; it has no co-ordination with the normal tissue; it is an independent, autonomous, uncontrolled growth of a tissue containing a mass of aberrant or abnormal cells.

- Cancers now cause the 2nd largest number of deaths in most countries.

- Cancer is a genetic disorder in which the normal control of cell growth is lost.

- At the molecular level, cancer is caused by the Mutation(s) in DNA, which results in aberrant cell proliferation.

- Different terms such as oncogenes, proto-oncogenes, tumor suppersor genes, viral oncogenes etc are used to explain the mechanism of cancer.

- Proto-oncogenes - Proto oncogenes are widespread in vertebrates and metazoa-from human beings to fruitflies.
- They are well conserved in their genomes , suggesting that they serve some essential functions in normal cells.

- Proto oncogenes undergo mutations to form oncogenes.
- Proto oncogenes activated to form oncogenes by mutation or over expression

- Oncogenes isolated from cancer cells-cellular oncogenes(c-onc)
- Viral oncogenes (v-onc)
- Viral oncogenes eg:V-src, V-ras, V-myc, V-mos

Viral oncogenesis

- The discovery that a prophage can exist in a cell for a long period of time suggests a similar possible mechanism for the viral origin of cancer.

- If a prophage can exist in a bacterial cell and at some point alter the expression of the cell's DNA, this could explain how animal viruses cause malignant changes. E.g. viral genes inserted into a human chromosome could disrupt regulation of some genes, allowing structural genes to be active at the wrong times, continuously, or maybe even not at all.

- Fetal genes (embryonal genes) cause rapid proliferation of cells during early development, but growth soon slows down and eventually stops in adulthood. If these fetal genes were turned on again later in life in a group of cells, they could rapidly grow into a tumor.

Viral oncogenes

- Dr. Francis Peyton Rous(1911) extracted tumor cells from hen breast tumor.

- Fowl sarcoma caused by a RNA virus -Rous Sarcoma Virus(RSV)
- virus is an infectious etiologic agent of cancer.

- Viruses causing tumors in animals -1st demonstrated by Shope.
- 10-20% of human cancer worldwide have a known viral etiology

- Viruses that produce tumours in their natural hosts or in experimental animals or induce malignant transformation of cells on culture are known as oncogenic viruses.

- The region of the viral genome which is a gene that encode proteins triggering transformation of normal cells into cancer cells is- viral oncogene(v-onc).
- About 20 viral genes are oncogenes

Oncogenic Viruses

RNA viruses

Retro viruses- Human T cell leukemia viruses

DNA viruses-Human Papilloma Virus(HPV)

Hepatitis B Virus

Mechanisms of viral oncogenesis

- Exact mechanism of viral oncogenesis are not well understood
- Viral oncogenes are responsible for oncogenesis resulting from persistent virus infection
- They transform normal host cells into cancer cells-integration of their RNA or DNA portion with host genome.
- It is a multistep process and may be partial or complete
- In the case of oncogenic DNA viruses, viral DNA(or a portion) is integrated with host cell genome.

- A virus transformed cancer cell is in many ways analogous to a bacterium lysogenised by a defective phage. In both cases, the cell is not destroyed and no virus is produced.

- In general, retro viruses induce tumours by 2 mechanisms- either by introducing a new transforming gene(oncogene) into the cellular genome or by inducing or altering the expression of a pre existing cellular gene.

- Mechanism of conversion of proto oncogenes into oncogenes by viral oncogenes

- 1. rapid replication of viral gene stimulates rapid expression of proto oncogene next to it.
- 2. proto oncogene may mutate to oncogene
- 3. the proto oncogene might shift from its normal position to a gene which is normally very active

  e.g. In cancer of white blood corpuscles
- 4. inactivation of tumour suppressor genes leads to cancer formation.

THANK YOU