Plasmid

A plasmid is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. They are most commonly found in bacteria as small, circular, double-stranded DNA molecules; however, plasmids are sometimes present in archaea and eukaryotic organisms. In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance. While the chromosomes are big and contain all the essential information for living, plasmids usually are very small and contain only additional information. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms.

Plasmids are considered *replicons*, a unit of DNA capable of replicating autonomously within a suitable host. However, plasmids, like viruses, are not generally classified as life. Plasmids can be transmitted from one bacterium to another (even of another species) via three main mechanisms: transformation, transduction, and conjugation. This host-to-host transfer of genetic material is called horizontal gene transfer, and plasmids can be considered part of the mobilome. Unlike viruses (which encase their genetic material in a protective protein coat called a capsid), plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host. However, some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer. The size of the plasmid varies from 1 to over 200 kbp, and the number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances.

The relationship between microbes and plasmid DNA is neither parasitic nor mutualistic, because each implies the presence of an independent species living in a detrimental or commensal state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or the proteins produced may act as toxins under similar circumstances, or allow the organism to utilize particular organic compounds that would be advantageous when nutrients are scarce.

Prion

Definition

Prions (the name is derived from proteinaceous infectious particle) is the name used by many scientists to describe the pathogen that causes **transmissible spongiform encephalopathies (TSE)** which are neurodegenerative diseases in mammals. **Prions are a disease-causing form of a normal protein called cellular prion protein (PrP^C) that is located primarily on the surface of central nervous system cells but also in other tissues of the body in mammals. The specific function of the normal prion protein (PrP^C) is not clearly understood, but in experimental models it appears to play a role in protecting cells and helping them respond to oxygen deficiency**.

Prions are extremely small, smaller than viruses, and even through an electron microscope only aggregations (clusters), not individual prions, can be seen.

Prions are unique pathogens in that they appear to have no nucleic acid and thereby differ from viruses, bacteria, fungi and other pathogens. Prions are resistant to procedures that break down nucleic acid and destroy biological forms of pathogens.

In addition, prions differ from other pathogens in that they are responsible for genetic, sporadic and acquired forms of neurodegenerative disease. Also, **because prions are an abnormal form of a normal protein that is genetically encoded, they do not produce an immune response in the host as would a foreign infectious agent**.

Lacking nucleic acid , prions cannot reproduce, but they replicate by stimulating normal cellular prion protein to refold into a form called PrP scrapie (**PrP^{Sc}**) – named after **scrapie**, the first TSE discovered. The conversion of normal prion protein (PrP^C) into abnormal prion protein (PrP^{Sc}) and replication of prions in the brain causes degeneration of neural tissue and, ultimately, death. The process by which the prion recruits normal prion protein (PrP^C) to convert to the disease-causing form remains unknown.

Prion Discovery

The similarities of certain animal and human TSE was noted beginning in the 1950s. Research published in the late 1960s demonstrated that the agent that causes the sheep TSE called scrapie was extremely resistant to deactivation by ultraviolet (UV) and ionizing radiation[2], treatments that would destroy anything with nucleic acid. However, the nature of the infectious agent remained a mystery and suggestions included small DNA viruses, membrane fragments, **polysaccharides** and proteins. Some scientists theorized that scrapie was caused by an agent that did not depend on nucleic acid for its ability to reproduce.

In 1982, Stanley Prusiner, M.D., of the University of California-San Francisco announced in the journal *Science* that he had succeeded in purifying the scrapie disease-causing agent and had identified it as a protein. In the journal article, Prusiner noted that "because the novel properties of the scrapie agent distinguish it from viruses, **plasmids**, and **viroids**, a new term "prion" was proposed to denote a small proteinaceous infectious particle which is resistant to inactivation by most procedures that modify nucleic acids." In 1997, Prusiner was awarded the Nobel Prize for his discovery.

While the prion theory dominates the science of TSE, other theories exist about the cause. These postulate that the real infectious agent does have DNA and includes a theory that a slow acting virus is the cause based on finding tiny virus-like particles in neural tissue associated with TSE. Another theory is that TSE are caused by virinos — hypothetical particles consisting of nucleic acid in a protective coat of host cell proteins. A bacterial theory proposes that a tiny, wall-less bacteria called spiroplasma attaches to normal prion protein in the brain and causes it to misfold.

Prion Strains

Different strains of prions cause different diseases. Strain-typing research conducted in England, Scotland and elsewhere has found the prions that cause **Creutzfeldt-Jakob disease (CJD)** in humans, **bovine spongiform encephalopathy (BSE)** in cattle,**chronic wasting disease (CWD)** in deer and elk, and scrapie in sheep each have distinct attributes. However, the infectious agent that causes BSE appears identical to the agent that causes **variant CJD (vCJD)** in humans.

Prions and Disease

When prions are present in the brain, they replicate by inducing normal prion protein understood and is the subject of extensive research. The normal prion protein structure is believed to consist of a number of flexible coils called alpha helices. In the abnormal form of the protein, some of these helices are stretched out into flat structures called beta sheets . The normal protein is broken down by cellular enzymes called proteases but the abnormal protein shape is resistant to these enzymes. As a result, as prions replicate, they are not broken down by proteases and accumulate in brain tissue.

Prions' Pathway to the Brain

The accumulation of prions in the brain causes neuronal cells to die and, in some types of TSE, a type of protein called amyloid accumulates in plaques, or flat areas, and causes degeneration of brain tissue. Recent research suggests prions disrupt the normal cell process of protein recycling which causes a buildup of faulty proteins and causes the death of the cell. The destruction of neural cells causes tiny holes in the brain tissue and a sponge-like appearance under the microscope, thus giving rise to the term spongiform disease.

The pathway to the brain is a subject of significant research, but currently, no comprehensive answer exists. In TSE linked to consuming TSE-infected material, it is theorized that once prions are ingested, they are taken up by the lymphoid tissue that drains the gastrointestinal tract including **Peyer's Patches** and other nodes. Prions also have been found in tonsil, spleen and appendix. From the lymphatic system, research suggests that prions replicate, access and move through the autonomic nervous system to the central nervous system. Once in the brain, the higher concentration of cellular prion protein speeds up the replication process.

Prions also may enter lesions or wounds in the oral cavity and access the **vagus nerve** as a pathway to the brain.

Recent laboratory research using fluorescent dye to "brand" scrapie proteins has tracked prions as they invade nerve cells and then travel along wire-like circuits to points of contact with other cells.[9] This appears to be the way the prions that cause TSE invade nerve cells and are transported along neural circuits throughout the nervous system.

Tissue Infectivity

Prions typically are found in highest concentrations in tissues with high concentration of normal cellular prion protein, specifically neural tissue. However, the distribution of prions within the tissues of the body differs by species and by type of prion disease.

Deactivation of Prions

Prions are highly resistant to disinfectants, heat, ultraviolet radiation, ionizing radiation and formalin. However, prions can be deactivated by heat, by chemicals and by a combination of heat, chemicals, pressure and time.

Prions can be destroyed through incineration providing the incinerator can maintain a temperature of 900 F for four hours. In an autoclave, prions can be deactivated by using a temperature of 270 F at 21 psi for 90 minutes. If the infectious material is in a solution of sodium hydroxide, deactivation will occur after one hour at 250 F and 21 psi.

A commercial disinfectant called Environ LpH also has been shown to be effective at deactivating prions. Prion disinfection occurs with a 1 percent solution of LpH for 10 hours or with a 10 percent LpH solution for one hour.

Carcasses of infected animals can be deactivated into a sterile alkaline solution using an alkaline hydrolysis digester. This consists of an insulated steam-jacketed stainless steel vessel which operates at up to 70 psi and 300 F into which sodium hydroxide and water is added and heated and continuously circulated. This process degrades proteins into salts of free

amino acids and the temperature and alkali concentrations deactivate prions by destroying their peptide bonds.