Subcellular Life Forms

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1 Introduction

I like biology, but as a mathematician, I am drawn to the elegance of the very simplest forms of life: the subcellular life forms. They are so simple, in fact, that even calling them "alive" can be controversial. They lack many of the usual features of life. They don't have cell walls, most of them don't metabolize, and they are all parasitic, depending on other organisms for their ability to reproduce! Some of them even have no genetic code! Many of them cause diseases, but others are crucial to the well-being of their host, and many are so well integrated with their host that it becomes difficult to decide whether they are part of the host or a separate entity. Indeed, besides my love of elegance and my morbid fascination with parasites, the main reason subcellular life forms appeal to me is that they challenge our naive notion of organisms as entities with clear, well-defined boundaries. It's clear by now that life doesn't respect this simple picture. Whenever a pattern of any sort, however abstract, is able to replicate itself, it does! Typically these patterns overlap and interact in subtle ways, so one can't easily say where one ends and the other begins. These are the main kinds of subcellular life forms that I know about so far:

- Viruses
- Viroids
- Virusoids
- Plasmids
- Transposons
- Prions

I'll say a little about each kind. If you know any more fascinating facts about subcellular life forms - especially if you know kinds that aren't on this list - please email me! Some of the above terms are defined in an essay by Diener and Prusiner called "The Recognition of Subviral Pathogens" [8]. But beware! People argue quite a bit about the correct classification of these life forms. That's part of what's interesting about them: they really stretch our ideas in biology to the breaking point. One thing to keep in mind: these life forms are *small*. Remember that DNA is a double helix containing information in the form of AT and CG "base pairs" (paired molecules of adenine and thymine, or cytosine and guanine), while single-stranded RNA is a single helix containing information in the form of A, U, C, and G "bases" (molecules of adenine, uracil, cytosine and guanine). The human genome is made of DNA and contains about 5 billion base pairs. The genome of a bacterium is also made of DNA but has less than 10 million bases. The potato spindle tuber viroid, on the other hand, is nothing but a circular loop of RNA consisting of 359 bases! Small, simple - but effective!

2 Viruses

Diener and Prusiner define a virus to be a "small infectious pathogen composed of one or more nucleic acid molecules usually surrounded by a protein coat." They typically reproduce by latching onto the wall of a cell and inserting their genetic material - i.e., the nucleic acids - into the cell. This genetic material then uses the cell's machinery to make more copies of the virus. Typically, these copies overrun the cell until it bursts. However, the actual life cycle of a virus is often more complicated than this thumbnail sketch! Viruses use a large number of sneaky tricks to overcome the defense mechanisms of the cell. Apart from their intrinsic interest, viruses are important because they cause many diseases among humans, such as:

- the common cold
- influenza (the flu)
- measles
- rubella
- mumps
- warts
- chickenpox
- smallpox
- acquired immunodeficiency syndrome (AIDS)
- herpes
- hepatitis
- rabies
- poliomyelitis (polio)
- encephalomyelitis
- encephalitis
- yellow fever
- dengue fever

as well as diseases of domesticated animals and plants. There is standard taxonomy of viruses [3], [10] & [6], but I will content myself with a rough classification into the following 3 sorts:

- DNA viruses
- RNA viruses
- Retroviruses

2.1 DNA viruses

The genome of a DNA virus is a single molecule of DNA, either linear or circular, and usually double-stranded. Outside the host cell, this DNA is surrounded by a protein coat. There are 5 known families of DNA viruses affecting humans. The size and structure of the DNA viruses varies widely, from the small hepatitus B virus (HBV), whose round shell contains a circular DNA molecule with about 2,400 base pairs, to the large brick-shaped or ovoid pox viruses, which have a lipid coating and whose DNA has between 120,000 and 360,000 base pairs. Like retroviruses, some DNA viruses work their way into the nucleus of their host cell and then copy themselves into the host's DNA. An example is the hepatitus B virus, which occupies liver cells. This can cause tumors.

2.2 RNA viruses

The genome of an RNA virus is usually a single molecule of RNA, either linear or circular, but some contain up to a dozen molecules of RNA. Outside the host cell, this RNA is protected by a protein coat. Most viruses are RNA viruses. There are 13 known families of RNA viruses affecting humans. RNA virus range widely in morphology and size, with their genome containing anywhere from 1,700 to 60,000 nucleotides. (Actually the smallest one, the hepatitis delta agent (HDV), is quite different from all the rest. Like a virusoid, it is a circular loop of RNA that can only reproduce in cells infected by a helper virus, the hepatitus B virus. But unlike a virusoid, it affects animals rather than plants, it has its own protein coat, and its genome is bigger than that of a virusoid, having 1,700 nucleotides instead of a mere 350 or so. However, its genome is much smaller than that of any other virus.) One can broadly classify RNA viruses into:

- positive-strand RNA viruses
- negative-strand RNA viruses
- double-stranded RNA viruses

A "positive-strand" RNA virus consists of single-stranded RNA that functions directly as messenger RNA in the host cell, so that ribosomes in the host cell synthesize various proteins needed by the virus when encountering this RNA. A "negative-strand" RNA virus consists of single-stranded RNA that does not function as messenger RNA, since it contains the complementary base pairs. Negative-strand RNA viruses carry enzymes with them into the host cell to synthesize messenger RNA from the RNA in the virus. "Double-stranded" RNA viruses have both positive and negative strands. For some reason these are more likely to consist of several separate pieces of RNA.

2.3 Retroviruses

Retroviruses are like RNA viruses when outside the host cell, but once inside the cell's nucleus, they can copy themselves into the DNA of the host cell using an enzyme called "reverse transcriptase", which translates RNA into DNA. They are thus intermediate between RNA viruses and nuclear DNA viruses. Once they are integrated into the DNA of the host cell, they may take a long time to reemerge. In fact, so-called "endogenous retroviruses" can be passed down from generation to generation, indistinguishable from any other cellular gene, and evolving with their hosts! The very distinction between host and parasite becomes somewhat blurry in this case. In fact, once an endogenous retrovirus lost the genes that coat for its protein coat, it would become indistinguishable from an LTR retrotransposon - one of the many kinds of "junk DNA" cluttering up our chromosomes. It has been estimated that between .01% and .1% of the genome of wild and laboratory mice consists of endogenous retroviruses. The same is probably true for humans. to form protein coats - since most mammalian DNA serves no known purpose, the above figures may be drastic underestimates. Indeed, 97% of human DNA is so-called "junk" DNA of this sort! Retroviruses are important in genetic engineering because they raised for the first time the possibility that RNA could be transcribed into DNA, rather than the reverse.

In fact, some of them are currently being deliberately used by scientists to add new genes to mammalian cells. In addition, retroviruses are important because AIDS is caused by a retrovirus: the human immunodeficiency virus (HIV). This is part of why AIDS is so difficult to treat. Most usual ways of killing viruses have no effect on retroviruses when they are latent in the DNA of the host cell. Many retroviruses cause tumors in animals. These viruses contain host-derived genetic information.

3 Viroids

A viroid is defined to be a "small infectious pathogen composed entirely of a low molecular weight RNA molecule". Thus, unlike a virus, a viroid has no protein coat. It is nothing but a single-stranded circular loop of RNA! Most viroids consist of about 250 to 575 nucleotides, much smaller than a typical virus. Also, viroids don't function as messenger RNAs, so they don't make the cell synthesize enzymes: they rely completely on pre-existing enzymes in the host for their reproduction. Most known viroids cause diseases in plants. The first viroid was discovered in 1971, by Diener. It's called the potato spindle tuber virus (PSTV), since it causes a disease that makes potatos abnormally long and sometimes cracked. At the time, Diener's isolation of the viroid causing this disease met with some skepticism, since it was so much smaller than any known virus. By 1991, however, at least 15 plant diseases had been traced to viroids. There are also 2 viroids known, the hop latent viroid (HLV) and a viroid living in grapevines, that cause no known symptoms! This raises the fascinating possibility that there could be more such viroids lurking around. The complete molecular structure of many viroids has been worked out, which has allowed a classification of viroids on the basis of their RNA sequences. Roughly speaking, there are a large family of viroids that share many features with PSTV, together with one viroid that seems very different: the avocado sunblotch viroid (ASBV). McInnes and Simons have proposed a further classification of the PSTV-type viroids into three kinds [9]. It is clear from these RNA sequences that viroids are not "degenerate viruses", as had once been thought. They are quite different from any known viruses. One interesting theory is that they arose from RNA that escaped from cell nuclei. It's also interesting that all viroid diseases have been detected in the 20th century, some quite recently - in contrast to diseases caused by viruses. Also, many viroid diseases have been spreading after their discovery, often due to human activity. A fascinating example is the coconut cadang-cadang viroid (CCCV), a disease of coconuts which has been spreading throughout the Phillipines. On the island of Luzon, a puzzling feature of this disease was that it only affected crops owned by speakers of Bicalano, while adjacent crops owned by speakers of Tagalog went unharmed! Eventually people realized that the viroids were spread by workers cutting the palms. Tagalog owners prefer to hire Tagalog workers, while Bicalanos hire Bicalanos, some of whom came from an area where the disease was prevalent. (See the article by Maramarosch entitled "The Cadang-Cadang Viroid Disease of Palms" [4].) Because of this sort of epidemiology, Diener has suggested that viroids may be latent to their native host plants (like HLV), becoming pathogenic only when transferred to other species thanks to agriculture. Indeed, the viroid causing tomato "planta macho" disease in Mexico, TPMV, has also been found in wild plants there, where it seems sometimes "recover" from ASBV by sending up a new shoot. This new shoot is still infected with the viroid, but it shows no symptoms other than reduced fruit yield. Descendents of such a "recovered" tree are also infected with the viroid, and also symptomless, except for reduced fruit yield. Thus the avocado appears able to "come to terms" with the viroid in some way. Personally, I'd like to raise this possibility: that some viroids actually play a beneficial role in their native host plants! This may seem surprising, but when we compare the behavior of plasmids, it may seem less so.

4 Virusoids

A virusoid is a "viroid-like RNA encapsidated in a virus shell that also contains viral RNA". In other words, like viroids, they are circular loops of RNA, usually containing about 350 nucleotides. But unlike viroids, they reside inside the protein coat of a "helper virus". They can only reproduce in cells that have been infected by this helper virus, because they use some of the RNA of the helper virus to reproduce. The helper virus is typically an RNA virus consisting of about 4500 nucleotides. In short, a virusoid is a parasite of its helper virus. But it's not always so simple. Sometimes the helper virus is unable to reproduce unless the virusoid is present! Then we have symbiosis rather than parasitism. The first virusoids were discovered in the early 1980s in Australia, associated with viruses causing plant diseases such as velevet tobacco mottle (VTMoV), solanum nodiflorum mottle (SNMV), lucerne transient streak (LTSV), and subterranean clover mottle (SCMoV). An interesting theory about the origin of virsoids is that in plants infected with both viruses and viroids, the viroids got encapsidated in the viruses and later lost their ability to reproduce independently. At this point, I should admit that the terminology concerning virusoids is quite confusing to me. People sometime use "satellite RNA" as a synonym for "virusoid, but I'm not always sure when it's supposed to be an *exact* synonym. Diener and Prusiner define a "satellite RNA" to be a "small RNA that does becomes packaged in protein shell made from coat proteins of another, unrelated, helper virus, on which the satellite RNA depends for its reproduction". The similar-sounding term "satellite virus" appears to be reserved for an RNA virus that depends for its reproduction on an unrelated helper virus, but whose genome codes for its own protein coat.

5 Plasmids

A plasmid is a "small autonomously replicating circular molecule of DNA that is devoid of protein and not essential for the survival of its host". Plasmids range in size greatly, from about 4350 to 240,000 base pairs. Most known plasmids infect bacteria, but some infect plant and animal cells. They often copy themselves into the DNA of the host cell, and many carry genetic traits from one cell to another. Most plasmids keep a limit on the number of copies of themselves they keep in each host - the so-called "copy number", which ranges from 1 to about 40. Many plasmids are "conjugative". This means they can transfer copies of themselves from one host to another by forcing the host to undergo "conjugation" - a form of sex in which genetic material is exchanged between bacteria. People tend not to speak of plasmids as “life forms” quite as often as they do with viruses. In part this may be because plasmids are sometimes beneficial to their host cells, rather than pathogenic. However, is difficult for me to resist the impression that plasmids are just as “alive” as viruses. Indeed, some viruses become plasmids when parts of them are missing! For example, the “lambda bacteriophage” is a virus that infects the intestinal bacterium E. coli, but “lambda dv particles”, which arise from the lambda phage simply by deleting some DNA, are plasmids. The lambda phage multiplies inside its host and then kills it by “lysis”, which destroys the cell membrane and releases lots of copies. The lambda dv particles, on the other hand, stays in the cell in a fairly stable number of copies and does not kill its host. The difference is that while the lambda dv particles contain genes for replication, they lack genes for lysis and the protein coat. If we think of plasmids as life forms, we must admit that they are very successful. Many plasmids spread so thoroughly in cultures of bacteria that less than one cell in 100,000 lacks a copy! Some kinds of plasmids contain genes that help make sure copies are efficiently passed on to both daughter cells when the host cell divides. F plasmids have a particularly clever mechanism - they temporarily inhibit cell division when they have not yet replicated inside the host! Plasmids are diverse and very interesting. Some important kinds are:

- R Plasmids
- F Plasmids
- Colicin Plasmids
- Virulence Plasmids
- Metabolic Plasmids
- Tumor-Causing Plasmids
- Cryptic Plasmids

While they don’t quite fit under this heading, I can’t resist also mentioning

- Cosmids
- Phasmids

These are man-made entities based on plasmids, used in biotechnology. Are they alive? You judge. Some good books on plasmids include “Plasmids” by Paul Broda [1], “Bacterial Plasmids” by Kimber Hardy [7], and “Plasmids of Eukaryotes: Fundamentals and Applications” by K. Esser et al [5].

5.1 R Plasmids

R plasmids were first discovered in Japan in 1957. In Japan, dysentary was treated with sulphonamide until about 1950. Then, more and more strains of the bacteria causing dysentary became resistant to this antibiotic, rapidly rendering it ineffective. Doctors then began using tetracycline, streptomycin and chloramphenicol. By 1957, 2% of the bacteria causing dysentary were resistant to one of more of these drugs, and by 1960, 13% were resistant. It turned out that R plasmids were the culprit! R plasmids contain genes that give their bacterial hosts resistance to antibiotics as well as to poisonous metal ions such as arsenic, silver, copper, mercury, lead, zinc and so on. Because many R plasmids are conjugative, this resistance can spread from one bacterium to another. Because they can live in more than one species of bacteria, R plasmids can also spread resistance between bacteria of different species! Spread of resistance to antibiotics is now a major problem in medicine. Drugs which were used for many years to control bacterial diseases are now becoming helpless against new resistant strains. The problem has been made worse by the tendency for doctors and veterinarians to use antibiotics when they aren't strictly necessary, for example as part of livestock food. As a result an environment is created where bacteria with resistance have a great competitive advantage, so they spread rapidly. It has also recently been found that weeds growing near crops that were genetically engineered to resist herbicides can acquire this trait. I'm not sure, but I suspect that this happens via plasmids as well. R plasmids make it clear that the idea of evolution as a battle between species with separately evolving genomes is a great oversimplification. Instead, genetic communication and cooperation between different species can be very important.

5.2 F Plasmids

F plasmids live in the bacterium E. coli and were discovered in the 1920s. An F plasmid contains genes that make the cell membrane of its host form long tubes. These tubes, called "sex pili", attach themselves to other E. coli and puncture their cell membranes. The F plasmid then duplicates and a copy passes from the original host to the new host. A clever system has evolved to ensure that the sex pili of a given bacterium never attach to itself. F plasmids give their hosts no known traits besides these sex pili. The evolutionary origins of sex are much debated these days; we see here the fascinating possibility that sex can originate as a kind of disease whose sole function is to spread a parasite!

5.3 Colicin Plasmids

Colicin plasmids contain genes that give their host bacterium a certain small probability of bursting open and releasing chemicals called "colicins". These chemicals kill other bacteria by rendering their cell membranes permeable to important ions. There are many strains of colicin plasmid. Each one confers immunity only to the particular sort of colicin *it* produces. Different strains of colicin plasmid are "incompatible", meaning that a given strain bacterium cannot stably contain both. In short, different strains of colicin plasmid compete with each other using the resources of their hosts. A colicin plasmid will confer an advantage to its host bacteria if the other strains of bacteria nearby do not have a colicin plasmid. However, when there are many different strains of colicin plasmid present, all strains of host bacteria suffer. Thus there is a certain similarity between colicin plasmids and "protection rackets" run by Mafia-like gangs. Colicin plasmids are not the only sort of plasmids that exhibit incompatibility. Similar plasmids tend to be incompatible with each other, while drastically different plasmids are usually compatible. One theory is that incompatible plasmids use the same mechanisms to maintain their copy number. In a cell containing two incompatible sorts of plasmid, their reproduction is blocked until the total number of copies of the two together drops to the copy number of each one. This is an unstable situation, especially for plasmids with a low copy number, so eventually descendants of the host cell contain only one or the other plasmid.

5.4 Virulence Plasmids

Virulence plasmids contain genes that make their bacterial hosts more virulent to *their* hosts. A familiar example involves the bacterium E. coli, which inhabits the human large intestine. Certain strains of E. coli contain plasmids whose genes make the E. coli synthesize toxins that cause diarrhea. These "enterotoxigenic strains" of E. coli are probably an important cause of diarrhea among travellers. More seriously, in developing countries, diarrhea is one of the principal causes of death among those under five. "Vibrio cholerae", the cause of cholera, is a bacterium whose genes code for a diarrhea-causing toxin. The DNA of these genes is closely related to the DNA of certain virulence plasmids infecting E. coli - so closely that there is almost certainly a common ancestor. For example, Vibrio cholerae could have evolved from an earlier bacterium by permanently integrating the DNA from a virulence plasmid into its genome. Strains of bacteria and viruses often become less virulent as they coevolve with their hosts. Thus one may wonder what evolutionary advantage a virulence plasmid could confer to the bacteria containing it. In the case of bacteria causing diarrhea, there is an obvious possibility: diarrhea can serve as a mechanism for spreading the bacteria - and their plasmids - that cause it!

5.5 Metabolic Plasmids

Metabolic plasmids contain genes that let their bacterial hosts metabolize or degrade otherwise indigestible or toxic chemicals. For example, the bacterium Pseudomonas putida is able to grow on a wide range of organic compounds that are toxic to most bacteria, including toluene, octane, camphor, napthalene and nicotinic acid! It does this with the help of genes contained by metabolic plasmids called TOL, OCT, CAM, NAH and NIC plasmids. It's worth noting that some of these chemicals are secreted by plants as part of a defense against bacteria. Thus we probably have a kind of natural chemical arms race going on here. Other metabolic plasmids allow bacteria to degrade herbicides like 2,4-D, as well as certain detergents! People are investigating the use of such plasmids to help biodegrade pollution.

5.6 Tumor-Causing Plasmids

"Crown gall" is a cancer of plants caused by a bacterium known as Agrobacterium tumefaciens. But actually, the disease is caused by a plasmid having this bacterium as its host! When the plasmid passes from the bacterium to the cells of infected fruit trees, some of the genes contained in the plasmid cause tumors. Do these tumors help spread the bacteria to other trees?

5.7 Cryptic Plasmids

Cryptic plasmids are plasmids that have no known effect on their hosts. How much of this is our ignorance, and to what extent is being truly "cryptic" a successful strategy?

5.8 Cosmids

Cosmids are man-made circular loops of DNA containing plasmid DNA together with an arbitrary sequence of up to 45,000 base pairs of DNA. They are constructed by recombinant DNA techniques and then packaged in lambda phage protein coats. They are used to transfer genes to bacteria. The lambda phage is a virus that specializes in invading bacteria such as E. coli. In nature, its protein coat latches onto the bacterial cell membrane and injects the phage DNA into the bacterium. Biotechnologists have taken advantage of this by using the lambda phage protein coat to inject a cosmid into the bacterium! Once inside, the cosmid replicates like a plasmid and, like a plasmid, integrates its DNA into the genome of the bacterium.

5.9 Phasmids

Phasmids are man-made linear DNA molecules whose ends are sequences taken from the lambda phage, while the middle is a sequence taken from a plasmid, together with a sequence of whatever DNA one wants. Like cosmids, they are constructed by recombinant DNA techniques and packaged in lambda phage protein coats, and used to transfer genes to bacteria. However, both the lambda phage and plasmid replication functions are intact. In particular, they contain the lambda phage genes for "lysis", the process whereby a virus dissolves the cell membrane of its host. Depending on the conditions, the phasmid can act either like a phage or a plasmid - hence its name.

6 Transposons

Transposons, or "transposable elements", are sequences of DNA that move within their host's genome from one position to another. They were first discovered in the 1940s by Barbara McClintock, who later won the Nobel prize for this work. They exist in all known organisms, often in large quantities. Their main "function" appears to be simply their own self-replication, rather than any benefit to the host, or even any direct effect whatsoever on the host phenotype. For this reason, people sometimes refer to transposons as "selfish DNA". In addition to transposons, there is plenty of other DNA in our chromosomes that doesn't seem to code for proteins. This is sometimes called "junk DNA". It comes in various distinct forms, such as "introns", "satellite DNA", and "pseudogenes". In fact, junk DNA makes up about 97% of the human genome! Clearly despite its derogatory name, it's worth understanding and potentially very important. However, since transposons are the most "organism-like" of junk DNA, I will only talk about them here. There is a fair amount of genetic evidence that transposons spread "horizontally" between sexually isolated species in addition to being "vertically" passed down the evolutionary tree. However, the mechanisms of this horizontal transmission are poorly understood. One interesting fact is that certain viruses, the baculoviruses, can pick up and accomodate transposons from their hosts. They have been proposed as a possible mechanism for horizontal transmission of transposons. The two main classes of transposons are:

- Retrotransposons
- DNA Transposons

The best book on transposons seems to be "Dynamics and Evolution of Transposable Elements", by Pierre Capy, Claude Bazin, Dominique Higuet, and Thierry Langin [2]. In this book, retrotransposons are called "Class I elements", while DNA transposons are called "Class II elements". They also discuss "Class III elements". This seems to be a grab-bag consisting of transposons that don't clearly fit into the other two categories. Examples include the "Foldback" elements in fruit flies, the "Tu" elements in sea urchins, and "MITEs", or "miniature inverted repeat transposable elements", which are found mainly in plants and fungi.

6.1 Retrotransposons

Retrotransposons copy themselves from one location in the host genome to another using an RNA intermediate, with the help of reverse transcription from RNA to DNA. A rough classification of retrotransposons divides them as follows:

- LTR (long terminal repeat) retrotransposons
- non-LTR retrotransposons
  - LINEs (long interspersed nuclear elements)
  - SINEs (short interspersed nuclear elements)

LTR retrotransposons are are 5000-9000 base pairs long and have "long terminal direct repeats" - repeating sequences of base pairs at both ends. Between these are the genes needed for transposition, which code for enzymes like reverse transcriptase (which copies RNA into DNA), integrase (which integrates the DNA into the host chromosome), and so on. In all these respects, LTR retrotransposons are very similar to retroviruses. The most important difference is that retrotransposons do not code for the proteins forming the viral protein coat. There seems to be some debate as to whether retrotransposons are retroviruses that have somehow lost their ability to code for a protein coat, or whether retroviruses are retrotransposons that have somehow *gained* this ability. Of course, the two possibilities aren't mutually exclusive! As the name suggests, non-LTR retrotransposons lack terminal repeats. They have been divided into LINEs and SINEs. LINEs have a characteristic adenosine-rich sequence at one end, and are generally 5000-8000 base pairs long, though truncated versions are common. They code for various enzymes such as reverse transcriptase and RNase. The genomes of higher animals and plants may have over 10,000 copies of LINEs. In fact, at least 15 percent of the human genome consists of LINEs! SINEs are usually shorter than 500 base pairs. The source of the enzymes needed for the mobility of SINEs is not yet known - but perhaps it is LINEs! Higher animals and plants may have over 100,000 copies of SINEs.

6.2 DNA transposons

DNA transposons mainly move using a cut-and-paste mechanism: they code for an enzyme called a "transposase" that catalyzes a process in which the transposon DNA is excised and reinserted elsewhere in the host genome. Thus RNA and reverse transcriptase plays no role in their life cycle.

7 Prions

Prions are small, proteinaceous infectious particles that contain no detectable nucleic acid of any form, but are transmissible among certain animals, where they cause fatal brain diseases. These particles are rod-shaped, about 165 nanometers long and about 11 nanometers in diameter, and they consist largely of a protein called PrPSc, having molecular weight 33,000-35,000. They are able to resist inactivation by boiling, acid (pH 3-7), ultraviolet radiation (254 nm), formaldehyde, and nucleases! They can be inactivated by boiling in detergents, alkali (pH ¿ 10), autoclaving at 132 degrees centigrade for over 2 hours, and denaturing organic solvents such as phenol. Stanley Prusiner won the Nobel prize for medicine in 1997 for his work on prions. His theory is that prions are a modified form of a protein naturally occuring in the brain (PrP), and that this modified form can arise from a cell mutation, but then spread by means of a kind of autocatalyzed chain reaction. This theory was initially very controversial, because all other self-reproducing biological entities appear to contain RNA or DNA. There are still many doubters. In the earlier literature prions are sometimes called "slow viruses", because of their slow effect. However, no virus has ever been associated with prion diseases. Prions have recently received a lot of publicity as the cause of "mad cow disease", technically known as bovine spongiform encephalopathy. Starting in the mid-1980s, this disease infected thousands of cattle in England, in part because they were being fed offal containing nerve tissue from sheep infected with a prion-caused disease called "scrapie". People got worried that eating meat from cows with bovine spongiform encephalopathy could cause a prion-induced brain disease in people. This caused an enormous uproar. There are already a number of prion-induced brain diseases in people, such as Creutzfeldt-Jakob disease (which occurs spontaneously in about one in a million people) and kuru (transmitted by means of cannibalism among the Fore tribe in New Guinea). There are also prion-induced brain diseases in mink, cats, deer and moose.

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