Proteins Rule!

Prion Biology and Diseases
Edited by Stan Prusiner
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The concept that nucleic acid, DNA or RNA, makes up the genetic material has dominated biology for half a century (Avery et al., J. Exp. Med. *93*, 137–158, 1944). This was only achieved after a long period of hot debate. During the first half of the twentieth century the prevailing idea was that proteins made up the genetic material. Eventually it became clear that the functional abilities of proteins are determined by nucleic acids. However, recently a number of diseases have appeared in the spotlight that don't seem to follow this basic dogma of modern biology.

The saga of the "prion" diseases started in 1730 when a disease in sheep called scrapie was first reported (Parry, *Scrapie Disease in Sheep—Historical, Clinical, Epidemiological, Pathological, and Practical Aspects of the Nature of the Disease*, Academic Press, London, 1983). Scrapie is an infectious disease that can be transmitted between sheep. Keen observations revealed a striking similarity between the neuropathology observed in scrapied sheep and that in patients suffering from kuru, a disease found among the Fore people of Papua New Guinea. Both diseases produced pathology remarkably similar to a rare class of neurological disorders in humans, the most prominent of which is Creutzfeldt-Jakob disease (CJD). These disorders are characterized by degeneration of the central nervous system, with a spongiform appearance of the brain parenchyma. In variable amounts amyloid plaques are observed which consist of protein aggregates that show green birefringence under polarized light after binding the dye Congo red. About one in a million people are diagnosed with CJD each year. One of the most puzzling features of CJD and related diseases is that they can manifest themselves as either sporadic, genetic, or infectious diseases. The attempts to explain these three facets of the disease within one model have generated a large amount of scientific controversy over the years.

The discovery that kuru, a fatal neurodegenerative disease of man, was infectious resulted in the awarding of a Nobel Prize to Carleton Gajdusek (Science *197*, 943–960, 1977). This discovery has been popularized in the book *Deadly Feasts* (Rhodes, 1997). The fact that the scrapie agent could be transmitted to rodents greatly enhanced its study. Brain fractions highly enriched in infectivity consist primarily of a protein called PrP (for proteinaceous infectious particle or prion) with no defined nucleic acids present (Prusiner et al., Cell *35*, 349–358, 1983). PrP is the major component of the amyloid plaques seen in the brains of patients. The absence of essential nucleic acids was also inferred from radiation experiments (Alper et al., Biochem. Biophys. Res. Commun. *22*, 278–284, 1966). These observations led to the notion that a protein could be an infectious entity; the implication that a protein can be a gene, i.e., carry genetic information, spawned much controversy.

The funcion of PrP is still unknown, and despite a large body of scientific data, some still doubt that PrP is the sole agent responsible for CJD and scrapie. One of the main problems is that no one has ever been able to purify the infectious agent to homogenity. Even the purest preparations still contain only one infectious unit per 100,000 PrP molecules. However, this does not disqualify the protein only hypothesis. Another challenge is the occurrence of prion "strains," variants of the disease with distinct characteristics. These characteristics are even maintained when the diseases are propagated in inbred mice. Strains can easily be accommodated when prion replication depends on the presence of a nucleic acid. However, PrP is capable of forming amyloid fibers, fibrillar protein aggregates rich in β sheets. Subtle differences in PrP oligomerization could affect pathogenic properties and thus would manifest themselves as different prion strains.

The protein only model, which predicts that PrP acts by itself without the involvement of a nucleic acid to cause CJD and scrapie, elegantly explains the sporadic, genetic, and infectious manifestations of these diseases. In this model, the sporadic cases are caused by spontaneous misfolding of the protein, the genetic cases are caused by mutations in the PrP encoding gene that predispose the protein into adopting the prion form, and the infectious cases are logically caused by inadvertent exposure to prion protein.

Support for the notion that proteins can be infectious entities came from a very different field. In 1994 prions were proposed to exist in the yeast *Saccharomyces cerevisiae* (Wickner, Science *264*, 566–569). Yeast does not encode a PrP homolog. The yeast prions were discovered as unusual nonchromosomal genetic elements. Based on genetic criteria it was proposed that a change can occur in the Ure2 or Sup35 proteins, allowing these proteins to convert the normal proteins into the same altered prion forms. A change in Ure2 protein results in the [URE3] element, whereas a change in Sup35 protein results in the [PSI^+] element. Using the sophisticated genetic and molecular biology tools available for the study of yeast it has become firmly established that the [URE3] and [PSI^+] elements are infectious proteins. The genetic criteria that confirmed the existence of yeast prions were also used to reveal a fourth prion, the [Het-s] element of the fungus *Podospora anserina* (Coustou et al., Proc. Natl. Acad. Sci. USA *94*, 9773–9778, 1997).

Once one accepts the fact that proteins can be infectious entities, the question becomes why have only so few been discovered? All identified proteins capable of forming prions aggregate in the prion state. However, it should be pointed out that aggregation is by no means synonymous with prion formation. Many proteins that are able to form aggregates in vivo do not cause infectious disease. Furthermore, protein aggregation is by no means the only mechanism by which infectious proteins could form. Any self-sustaining protein modification could result in the formation of an infectious entity. Also not every prion has to be harmful to its host. Whereas PrP, Ure2p, and Sup35p manifest themselves as diseases in the prion state, the [Het-s] prion carries out a physiological function in *P. anserina*. The *het-s* gene belongs to a group of genes that determine the ability of the fungus to distinguish self from non-self. In its

native state the HET-s protein allows fungal hyphae to fuse, whereas in the prion state the HET-s protein causes incompatibility with fungal strains expressing the HET-S protein. This so called "vegetative incompatibility" is thought to be a major mechanism used by fungi to limit the spread of fungal viruses. Interestingly, although the mammalian prion diseases are invariably fatal and currently untreatable, all the fungal prions can be cured. This is most easily accomplished with the yeast prions that can be cured from these cells by growth on low concentrations of guanidine hydrochloride. In addition cells can be cured of [PSI$^+$] by modulating expression of the heat shock protein HSP104. The fact that signals exist that allow cells to modulate prion maintenance suggests a whole new level of genetic regulation. For instance, protein-based heredity could actively prepare offspring for conditions encountered locally by the parents. It thus would be expected that many more prions will be discovered in the near future.

Against much opposition, Stan Prusiner has championed the protein only hypothesis, resulting in him being awarded a 1997 Nobel Prize (Science *278*, 245–251, 1997). In *Prion Biology and Diseases*, Prusiner has brought together an impressive amount of scientific data dealing with prions (a word he coined). Of the book's 17 chapters he is first author of six and coauthor of three others; the book thus carries a firm imprint of the Prusiner lab. Despite this, it is the most complete summary of this complex field currently available. This book is a must for anyone interested in the prion field. For those not familiar with the field, the book provides ample introduction. The book is an invaluable reference source combining clinical, epidemiological, genetic, and biochemical data. An exhaustive amount of material is presented dealing with everything from how to isolate prions to strategies for analyzing PrP structure. It contains detailed descriptions of the various human and animal prion diseases as well as a chapter on the fungal diseases. Perhaps because of the wealth of information, some subjects are a little snowed under. For instance, the ability to create protease-resistant PrP in vitro (Kocisko et al., Nature *370*, 471–474, 1994) could have been addressed in a separate chapter.

The last chapter of the book deals with biosafety issues. This is a timely and important subject as prion diseases have evolved from scientific novelties into real health risks. This transition is the result of an epidemic in Great Britain of a prion disease found in cows, bovine spongiform encephalopathy (BSE) or "mad cow disease." This outbreak was not only a devastating blow to the cattle industry, it also exposed a potentially dramatic danger in our modern food production. The mad cow epidemic is thought to have been caused by the practice of using slaughterhouse refuse as a dietary supplement for farm animals. This system allowed BSE prions to accumulate. But the BSE prion has not remained confined to cattle. Since the BSE outbreak, 52 unusual cases of CJD have appeared that indicate that the BSE prion has moved through our food supply and infected people. Because the disease has a long incubation time, the extent of human infection can not yet be estimated. As so much about prions is still unknown, governments have adopted a worst case scenario. For instance, U.S. blood banks have banned donations from Americans who have spent six months or more in Britain. Clearly much work remains to be done on this fascinating subject of infectious proteins.

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βAPtism

Molecular Biology of Alzheimer's Disease
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$120.00

The main title of the book, *Molecular Biology of Alzheimer's Disease*, catches the attention of readers interested in the basic roots of Alzheimer's disease (AD), but the subtitle *Genes and Mechanisms Involved in Amyloid Generation* more appropriately describes the contents. A variety of changes in the brain are found in AD, including the aggregation of the amyloid peptide Aβ in amyloid plaques, the aggregation of tau protein in neurofibrillary tangles, a decrease in neurotransmitters (e.g., acetylcholine), a reduction in energy metabolism, an increase in oxidative damage, and an increase in inflammatory reactions (activated microglia). The relationship between these phenomena is currently not well understood, and therefore different schools of thought have evolved that focus on different hypotheses. For the pathologist, the two most visible changes are the β-amyloid plaques and the tau-containing tangles; this divides the field into "βAP-tists" and "tau-ists." This book is written mostly by baptists for baptists; it is a collection of reviews by leading scientists in the field of amyloid and Alzheimer's research. The majority of the 18 articles deal with the properties of the two types of genes whose products are responsible for most of the familial forms of AD—the amyloid precursor protein (APP) and its proteolytic fragment Aβ, and the presenilins (PS-1 and PS-2). Although familial (inherited) forms of AD account for only a minor fraction of AD cases (in contrast to the more common "sporadic" forms of AD), they have had an enormous impact on Alzheimer research because they point the way to one of the probable culprits, the Aβ peptide. The "amyloid hypothesis" of AD states that the pathological accumulation of Aβ causes the neurodegeneration in AD. It is remarkable indeed that diverse mutations in APP or in the presenilin genes all point in this direction, even though the molecular effects may be diverse. The Aβ peptide (∼40–42 residues) is cleaved out of the APP molecule by two proteolytic activities, termed β-secretase (N terminus) and γ-secretase (C terminus). Mutations in APP can cause enhanced production of Aβ, enhanced cleavage at the β- and γ-secretase