ARTICLES

Prion Infection Dynamics

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Propagation of a modified form of the cellular prion protein is thought to be the primary cause of the transmissible spongiform encephalopathies, which include kuru, Creutzfeldt-Jakob disease (CJD), scrapie, and bovine spongiform encephalopathy (BSE). These highly unusual neurological maladies seem to arise spontaneously at extremely low rates. In addition, these diseases can be transmitted directly, in which case the incubation period is remarkably constant. The challenge is to understand these crucial features of prion diseases, without invoking the action of any viral agent. A simple model is developed in which the onset and progression of spongiform encephalopathies are explained by the kinetics of prion aggregate formation. Interestingly, ordered aggregations of proteins such as occurs in prion diseases are also associated with other neurological disorders such as Alzheimer's disease. Thus, insights developed about prion aggregation may have wide significance.

KEY WORDS: prion proteins, prion infection dynamics, transmissible spongiform encephalopathies, mathematical model, prion self assembly

Prion proteins have been linked to several rare and bizarre fatal diseases, including Creutzfeldt-Jakob disease (CJD), kuru,[1] fatal familial insomnia (FFI),[2] scrapie,[3–5] and bovine spongiform encephalopathy (BSE).[6] These diseases are neurological maladies in which brain function is destroyed as neurons die and the brain tissue develops sponge-like holes. The technical name for such diseases is transmissible spongiform encephalopathies (TSE). It was not known until recently that these diseases were transmissible, and the mechanisms underlying the pathology are still not worked out.[7] Apparently, prion proteins, which are present in healthy brains, are somehow converted to a pathological configuration that forms insoluble aggregates inside the brain.

The TSEs of animals and people have shared a parallel history, in which the causes of infection have remained initially mysterious, subsequently to become attributed to a slow virus and more recently identified with a modified form of the prion protein. The human disease kuru, which attracted much public interest, is thought to have been transmitted from infected corpses by cannibalism,[8] while the route of transmission of scrapie, a wasting disease of sheep and goats, has never been identified. Hadlow was the first to suggest that kuru and scrapie were related diseases,[9] and Gajdusek demonstrated their infectivity.

The recent epidemic of BSE (or "mad cow disease") in Britain has been attributed to feeding cattle with infected ruminant-derived protein. Around 170,000 infected cattle have died from BSE in the last 10 years.[10,11] A further 5,000 cases are expected to occur over the next 4 years, with the number of cases (exponentially) declining. It has been estimated that a total of about 1 million potentially contaminated cattle have entered the human food chain.[10] A new variant of CJD was found in the United Kingdom and is thought to be linked to BSE.[12–14] About 20 human cases have been reported so far.

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It is now agreed that all TSEs, irrespective of their host, involve the prion protein. The healthy prion protein (denoted as either PrP^{C} for cellular prion protein or PrP-sen for protease sensitive prion protein) is found concentrated in the tissues of the nervous system, bound to the surface of nerve cells. The precise function of the protein in healthy tissue remains unknown, and gene-knockout studies creating prion protein-free mice

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have failed to show any major effects of gene deletion.[15,16] The prion protein is encoded by a single exon of a single copy gene which remains highly conserved throughout the mammal class.[17,18] This gene is now known to be identical with the *sinc* gene, which was found to influence scrapie incubation periods in sheep.[19] The prion diseases are all characterized by an accumulation of a pathogenic, protease resistant form of the cellular prion protein known as PrP^{Sc}, which stands for scrapie prion protein,[20] or PrP-res, which stands for protease resistant prion protein.[24] PrP-res is partially digested by proteinase k to produce the smaller peptide, $PrP^{27–30}$, which remains infectious. $Prp^{27–30}$ is able to form amyloid fibrils in solution which accumulate as neurotoxic aggregates in the extracellular spaces. It remains uncertain whether amyloid production plays an essential role in prion pathogenesis, since amyloid is not observed in all histological examinations of PrP-res inoculated fatalities.[23,24] However, there is evidence to suggest that there is a positive correlation between the severity of disease and the extent of amyloid production.[26] Some recent work appears to separate prion infectivity from the amyloid properties of PcP-res.[26]

The mechanism by which PrP-sen is converted into PrP-res, and the subsequent formation of amyloid, remain essential missing pieces in the puzzle of understanding the progress of the prion diseases. Over the course of 20 years of research, no evidence for a scrapie-specific nucleic acid has been found, despite extensive efforts. Preparations of PrP-sen treated with ionizing radiation and other agents damaging nucleic acids have not altered their infectivity.[27] Conversely, the importance of the prion protein alone in determining susceptibility to different prion diseases and in influencing disease latency has been documented in both inherited and infectious cases and has received a great deal of experimental support.[28] As a result, the preferred hypotheses for transmission of TSEs have been those based on protein-protein interactions rather than nucleic acid-protein interactions, as would be required by a virus.

One of the key mysteries surrounding TSEs concerns how proteins alone can function as infectious agents. The first models of prion propagation were published by Griffith[29] in 1967, who suggested that prion replication involves a conformational change of a healthy form into a diseased form.[29] The diseased form produces aggregates which can break to give rise to new "condensing nuclei." This idea provides the basis for what later became known as the "prion hypothesis" and is similar to the nucleation-dependent aggregation mechanism championed by Caughey, Lansbury, and colleagues, in which aggregates of PrP-res propagate by assimilating PrP-sen monomers into a growing structure.[30–33] The infectious unit is the aggregate and conversion is synonymous with integration of PrP-sen into the aggregate. This model of prion replication is reminiscent of Penrose's ideas about how mechanical automata could be self-reproducing.[34] As an alternative mechanism of prion propagation, Prusiner[5] has suggested the heterodimer hypothesis. This model is also based on the idea of a conformational change of the healthy form (PrP-sen) into the pathogenic form (PrP-res), but does not require aggregate information. Instead, PrP-res and PrP-sen form a heterodimer, which then dissociates into two molecules of PrP-res.

One of the key mysteries surrounding TSEs concerns how proteins alone can function as infectious agents.

In the absence of conclusive experimental evidence for or against the heterodimer hypothesis vs. the aggregation hypothesis, we favor the aggregation hypothesis because it is supported by other examples of propagation of conformational change of proteins. The amino acid sequence of a protein is considered to be the sole determinant of the tertiary structure of enzymes (which are usually tightly folded monomers), but it is known not to be the case for proteins involved in supramolecular structure.[35,36] The well-studied self-assembly of flagellar filaments from monomeric flagellin represents such an example.[37] Under normal conditions, flagellin monomers, on their own, do not associate, but a fragment of a flagellum can act as a nucleation seed and induce the aggregation of monomers into polymers. Other examples include self-assembly of actin filaments, the morphogenesis of bacteriophages,[38] or amyloid proteins.

Amyloid proteins are a feature of a number of diseases in addition to those associated with the prion protein, including Alzheimer's disease (AD), diabetes, thyroid carcinoma, and Down's syndrome.[39] There are also similarities between the sequence at the C-terminus of the β-amyloid protein of AD and a partial sequence in PrP-sen/res. Significantly, this partial prion sequence has been shown to seed amyloid production.[40] None of the non-prion amyloid diseases are thought to be infectious under natural circumstances.

Supporting evidence for the aggregation hypothesis has also come from yeast research. [PSI+] is a protein found in budding yeast with a self-propagating ability.[41,42] The protein determinant of [PSI+] is sup35, and is converted into the pathogenic isoform [PSI+] producing highly ordered fibrous aggregates.[43] The prion gene and the yeast gene Sup35 are not homologues, and hence infectious self-propagation appears to have evolved independently in these two groups.

Eigen[44] has analyzed the chemical kinetics of prion propagation. He begins with the simplest model describing Prusiner's heterodimer hypothesis. PrP-sen is produced by the genetic machinery and has, like other proteins, a finite lifetime. In the normal, healthy host, PrP-res is produced at some low rate by spontaneous conversion of PrP-sen. Once present, PrP-res is produced at a faster rate by interaction between PrP-sen and PrP-res. Eigen[44] analyzes the dynamics of this system, showing that two qualitatively distinct outcomes are possible, depending only on the relative magni-

tudes of the rate constants. Essentially, if the death rate of PrP-res exceeds the catalytic conversion rate, the system tends to remain in the normal PrP-sen state, with any inoculation of PrP-res decaying. Conversely, if these rate constants have the opposite relative magnitudes, then there is always exponential growth toward the asymptotic equilibrium, with PrP-res predominating. However, in this event, it becomes virtually impossible to juggle the rate parameters to produce a knife-edge where most of us seem to be in the healthy PrP-sen state, with only a few unlucky individuals victimized by PrP-res. Eigen[44] concludes that a "linear autocatalytic" model, implementing the simplest version of the heterodimer hypothesis, will not work.

Eigen[44] goes on to explore two different and more complex models that depart from linear autocatalysis. One of these models is a "cooperative Prusiner mechanism"; the other is an aggregation mechanism. In both cases, there are hierarchies of clusters of PrP-res and there can be a threshold effect. If the concentration of PrP-res is low enough, we can maintain the normal, healthy PrP-sen state. However, an inoculation of PrP-res which exceeds some threshold can tip the system exponentially into runaway autocatalysis of pathogenic PrP-res.

The diversity of prion strains and their ability to breed true when serially passaged among hosts is one of the curiosities of prion infection, because it suggests that the information carried by the infectious particle does not reside in the sequence but in the structure. It is important to realize that strains are classified according to incubation periods (the time from inoculation to clinical diagnosis), by lesion profiles[45] (the spatial distribution of lesions in the brain), and by behavioral differences.[46] More recently strains have also been characterized by the protease cleavage sites of PrP-res.[47–49] The generation of new strains has been thought to occur during vertical transmission, either by the selective amplification of preexisting variants or the de novo mutation of new variants.[50] Assuming that the prion is not a virus-like particle capable of rapid mutation, the latter would be hard to explain. In light of the protein-only hypotheses, strain formation is best thought of as the product of heterotypic interactions between the initial, distinct PrP-res sequences and the homologous host PrP-sen sequence.[6] The newly emerged strain would then propagate using the host sequence exclusively.

In a recent study, Telling et al.[49] used transgenic mice, expressing the human prion protein, to see what happened when various strains of pathogenic human prions were transmitted to these animals. They found that extracts from the brains of FFI patients caused the mice to develop well-defined signs of disease close to 200 days after inoculation, inducing forming of a 19-kD PrP-res fragment after protease digestion, the same size as seen in the human patients. Extracts from the brains of familial or sporadic CJD victims took from 170 to 190 days to produce disease in the mice; this was associated with a 21-kD PrP-res fragment, in mice as in humans. The differences in the pattern of fragmentation are interpreted as arising from different foldings of the host prion protein induced by the different human prion strains. Therefore, the biological properties of prion strains seem to be encrypted in the specific conformation of PrP-res.

This paper presents various ideas on prion replication dynamics made explicit via mathematical models that describe amyloid formation and propagation. We present a mathematical theory of prion infection dynamics based on the assumption that formation and fragmentation of prion amyloids are essential components of prion replication. Subsequently, we embed this mechanism into a cell-to-cell spread of the infectious prion and discuss how our model relates to the long (and constant) incubation period of prion disease. Finally, we present some ideas on how different prion strains might breed true in the same animal host.

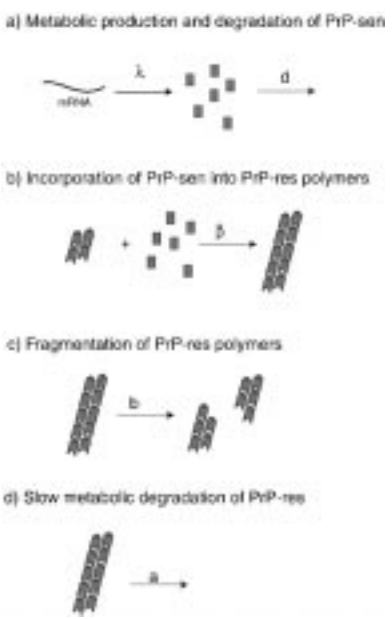

Figure 1. Prion replication dynamics may be based on the formation and fragmentation of ordered aggregates of PrP protein. **a:** We assume that the healthy protein conformation (PrP-sen) is produced at a constant rate, λ, by the genetic machinery of the host cell via translation of messenger RNA. PrP-sen is metabolically degraded at a rate d. **b:** PrP-sen monomers are incorporated into aggregates of PrP-res, which stands for the protease resistant, diseased form of the PrP protein. The change of conformation from PrP-sen to PrP-res is a consequence of aggregation. In the simplest model, aggregates grow at a constant rate, β. **c:** We assume that PrP-res aggregates can break into smaller pieces which then form new nuclei for aggregate growth. In this way, number of PrP-res aggregates increases. **d:** PrP-res aggregates are metabolically degraded at rate a, which is thought to be very slow compared to d.

PRION REPLICATION DYNAMICS

Figure 1 illustrates our assumptions about the chemical kinetics of prion propagation. The model distinguishes between healthy PrP-sen monomers and aggregates of PrP-res of various size. x denotes the abundance of PrP-sen protein and y_i, the abundance of PrP-res aggregates that contains i monomers. The total amount of PrP-res aggregates is given by $y = \sum y_i$.

We assume that normal PrP-sen protein is produced by the genetic ma-

chinery of the cell at a constant rate, λ. Like other proteins, it has a finite lifespan and therefore we assume that it is metabolically removed at rate *d*. The conversion of PrP-sen into PrP-res occurs by interaction between PrP-sen monomers and PrP-res aggregates. When a monomer is incorporated into the PrP-res aggregate, the size of the aggregate increases by one. The rate of this reaction is given by $\beta_i x y_i$. We also assume that PrP-res aggregates can break up. An aggregate of size *i* can break into two pieces of size *j* and *i* – *j*. This happens at rate $b_{i,j}$. Finally, we assume that PrP-res aggregates are metabolically degraded at rate *a* (which is small compared to *d*). These simple kinetics lead to the following system of ordinary differential equations:

$$\begin{aligned}
\dot{x} &= \lambda - dx - \sum_{i=1}^{\infty} \beta_i x y_i \\
\dot{y}_i &= \beta_{i-1} x y_{i-1} \beta_i x y_i - a y_i + \\
&\quad \sum_{j=i+1}^{\infty} (b_{j,i} + b_{j,i-j}) y_j - \\
&\quad \sum_{j=1}^{i-1} b_{i,j} y_i \qquad i = 1,2,\ldots.
\end{aligned} \tag{1}$$

Different assumptions about the dynamics of aggregate growth and fragmentation can be embodied in the structure of the vector β_i and the matrix $b_{i,j}$. The simplest such assumption, which we will pursue in this paper, is that aggregates break with equal probability at each site, and that all aggregates grow at a uniform rate, $\beta_i = \beta$.

Figure 2a illustrates the dynamics of this system. Initially, the total amount of PrP-res aggregates grows exponentially. During this phase the average aggregate size first increases rapidly to a certain maximum value and then remains constant. The abundance of PrP-sen, given by *x*, remains at its preinfection level, $x_0 = \lambda/d$. Eventually, *x* starts to de-

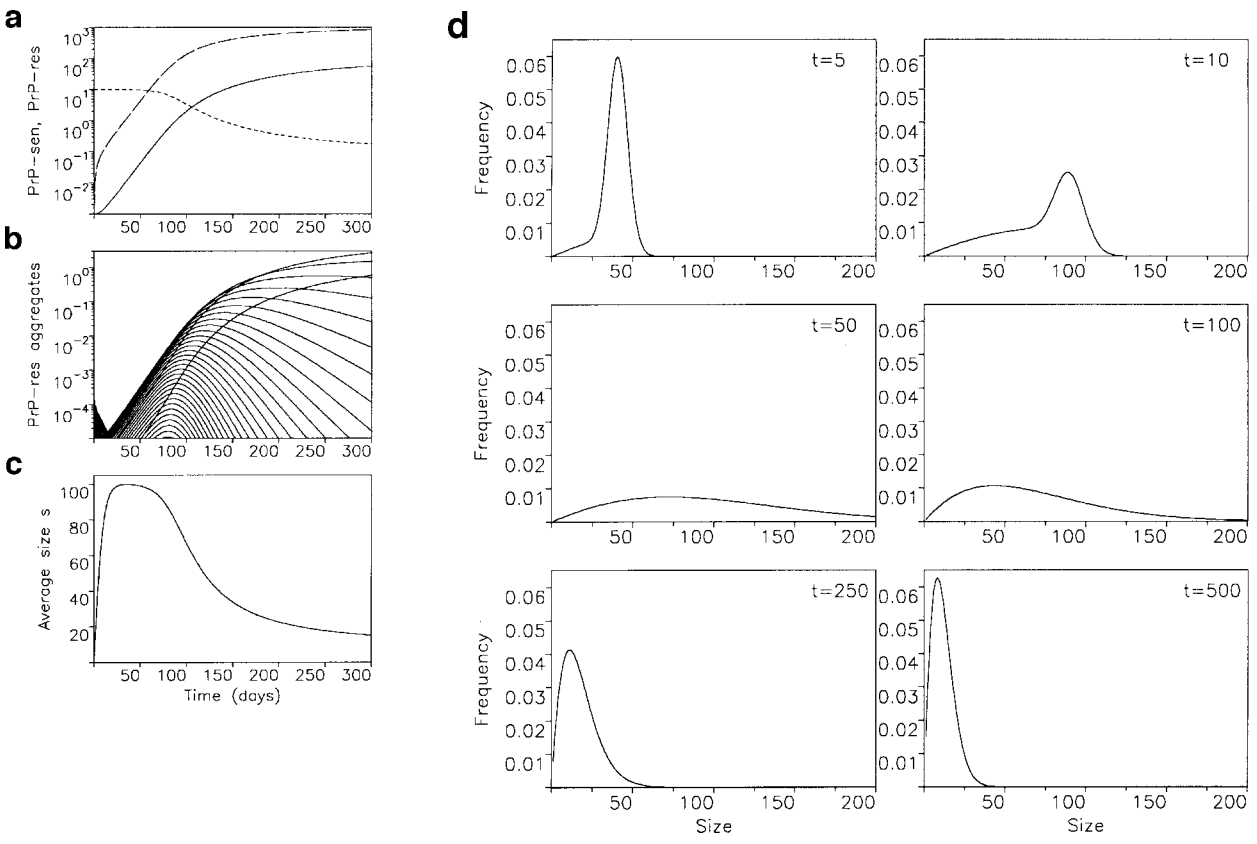

Figure 2. Dynamics of prion replication according to the aggregation mechanism described in Figure 1. **a:** The increasing continuous and broken lines indicate, respectively, the total number of PrP-res aggregates, *y*, and the total number of monomers bound in aggregates *z*. There is a slow exponential growth phase, followed by saturation. The decreasing, dotted line indicates the abundance of PrP-sen monomer. **b:** Dynamics of individual aggregate sizes, y_i. Every 10th aggregate size is shown, i.e., *i* = 1, 11, 21, ... **c:** Dynamics of average aggregate size, *s* = *z*/*y*. Initially, there is a rapid increase, then during most of the exponential growth phase the average aggregate size stays almost constant at a maximum level, and finally the average aggregate size declines toward a lower equilibrium value. **d:** Size distribution of PrP-res aggregates at 6 time points (days 5, 10, 50, 100, 250, and 500) during the infection process. The simulation is based on Eq. (A1). Parameters: λ = 10, *d* = 1, *a* = 0.01, β = 1, *b* = 0.001. Units: Time in days (corresponding to the time scale of a typical prion infection in mouse); prion abundance in arbitrary units.

cline, which causes the exponential growth of y to slow down; the average aggregate size declines. The system settles to an equilibrium where the abundance of PrP-res is much higher than the original, preinfection level of PrP-sen. (This is a consequence of the removal rate of PrP-res, which is much smaller than the removal rate of PrP-sen; $a << d$.) The average aggregate size settles to an equilibrium level which is lower than the average size during the exponential growth phase. This is because the concentration of PrP-sen at equilibrium is low and the rate of aggregate growth is reduced compared to the initial phase, while aggregates are still broken into pieces at the same rate.

Figure 2b shows the dynamics of aggregate size distribution during infection. Initially, there is a sharp distribution around a peak value which is close to the average size. As the average size increases, the width of the distribution increases, too. The average then reaches a maximum value while the variance is still increasing. Then the variance reaches a maximum level, too, while the average begins to decline. Both average and variance are then declining. At equilibrium we find again a rather narrow distribution of aggregate sizes, but this time around a lower average value.

Analytical insights into these dynamics are derived in Appendix A. There we show that the model can be fully described by a system of only three differential equations in the variables x, y, and $z = \sum_i i y_i$. Interestingly, this system is equivalent to the basic model of virus dynamics, where x, y, and z would denote uninfected cells, free virus, and infected cells.[51] In the current context, x, y, and z denote, respectively, the abundance of PrP-sen monomers, pathogenic PrP-res aggregates, and total number of monomers bound in PrP-res aggregates. In Appendix A, we show that there is a basic reproductive ratio[52] given by

$$R_0 = \frac{\beta x_0 b}{a(a+b)}. \qquad (2)$$

If $R_0 > 1$, then the prion infection can spread within the infected individual. Conversely, if $R_0 < 1$, then the prion cannot propagate.

An implicit assumption of the above model is that aggregate growth can be initiated by the monomer, y_1, and occurs at a uniform rate. Alternatively, it is more realistic to assume that aggregation requires a nucleation seed of a certain minimum size. The easiest way to introduce this concept into our model is by assuming that all aggregates below a critical size, n, are unstable and rapidly fall into pieces that convert back to PrP-sen.

We believe that nucleus formation is a time-limiting step in cases of sporadic or inherited prion diseases.

In Appendix B, we present an analysis of this more complicated system. The overall dynamics are very similar to the simpler model described by Eq. (1), if sufficient nucleation seeds of size n or greater are present in the system. However, if more pathogenic PrP-res aggregates are below size n, then the two models behave differently: the kinetics of nucleus formation can be slow and can generate an important bottleneck for prion spread. We believe that nucleus formation is a time-limiting step in cases of sporadic or inherited prion diseases. In experimental or natural transmissions of prion agents, it is likely that enough nucleation seeds are transferred into the new host such that nucleus formation is not a time-limiting step. There is, however, the possibility that only very small PrP-res aggregates may be taken up by uninfected cells and therefore nucleus formation could be a rate-limiting step whenever a new cell becomes infected.

DYNAMICS OF DISEASE PROGRESSION

The models we introduced in the previous section describe prion kinetics in a homogeneous medium. We now extend the framework of our model by including an underlying cellular structure of prion propagation in order to highlight some questions concerning the cell-to-cell spread of prions and the overall dynamics of disease progression.

Consider a number of cells randomly distributed on a two-dimensional array. Within each cell, prion kinetics are described by Eq. (1). We assume that each cell continuously releases pathogenic PrP-res aggregates of various sizes into the medium which can then infect neighboring cells. Specifically, we assume that the probability that a given cell becomes infected is proportional to the total number of PrP-res molecules in a neighborhood of radius, R.

Figure 3 shows a computer simulation of this process. Initially, one cell is infected. As PrP-res concentration increases in this cell the neighboring cells become infected. Figure 3a shows the spatial dynamics of prion propagation and Figure 3b the increase in total pathogenic PrP-res abundance and the increase in the frequency of infected cells as a function of time. Total PrP-res abundance grows faster than linear, but slower than exponential. The saturation of the growth curve is a consequence of all susceptible cells (in the region described by our simulation) eventually becoming infected.

Neglecting the complication of spatial spread, we can describe the above dynamics with the following very simple epidemiological equations:

$$\begin{aligned} \dot{x} &= -\beta x y_2 \\ \dot{y}_1 &= \beta x y_2 - a y_1 \\ \dot{y}_2 &= a y_1 - b y_2 . \end{aligned} \qquad (3)$$

Here, x, y_1, and y_2 denote, respectively, uninfected cells, infected cells which are not yet producing infectious prions, and infected cells which are producing infectious prions. Uninfected cells become infected at a rate proportional to infectious cells; the rate constant is β. Infected cells, y_1, turn into infectious cells, y_2, at rate a. Infectious cells, y_2, die at rate b. The total abundance of cells is given by $z = x + y_1 + y_2$. Thus, $1/a$ is the average time span between infection of a cell and start of releasing infectious material. This could be called the "latent phase."

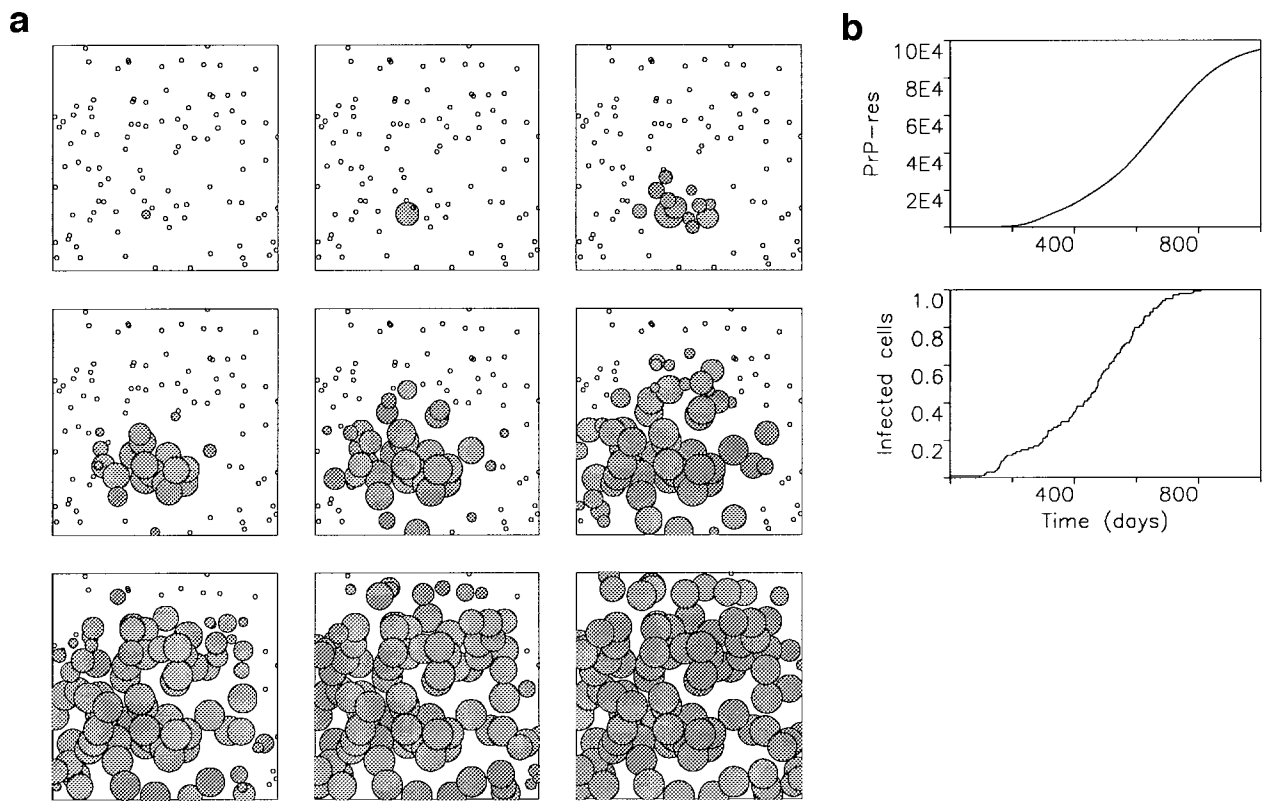

Figure 3. A cellular model of prion propagation. The hundred cells are randomly distributed on the unit square. Initially, one cell becomes infected. Within each cell, prion dynamics is described by Eq. (A1) with the same parameters as in Figure 2. The probability that the prion infection spreads to a neighboring cell is proportional to the total number of PrP-res aggregates within a cell (or, more realistically, on the surface of a cell). The proportionality constant is 0.001; cells within a radius 0.2 can become infected. **a:** Six consecutive time points are shown (day 0, 100, 200, 300, 400, and 500). Uninfected cells, open circles, infected cells, solid circles. The radius of an infected cell is proportional to the total amount of PrP-res in this cell. **b:** Sigmoidal increase in total PrP-res abundance and fraction of infected cells.

Whereas $1/b$ denotes the time span during which an infected cell releases infectious prions. This could be called the "infectious phase."

We are interested in the length of the incubation period of the disease, defined as the time between infection of an animal and first detection of symptoms. In terms of our model, given by Eq. (3), we can define the incubation period as the time between infection with prion and significant accumulation of cell death. A detailed analysis of Eq. (3), which represents a standard model of epidemiological dynamics, can be found in reference 47. We have to distinguish two limiting cases:

1. If $a >> b$, corresponding to the latent phase being short compared to the infectious phase of a prion cell cycle, we obtain as a rough approximation for the time t until a fraction f of cells have died $t \approx (1/b)(h_1 + h_2)$, where $h_1 = (1/R_0) \ln[z_0/y_0]$ and $h_2 = \ln[1/(1 - f)]$. Here, $R_0 = \beta z_0/b$ is the basic reproductive ratio of the cell-to-cell spread of the prion infection; z_0 and y_0 denote, respectively, the total number of cells and the number of infected cells at the beginning of infection ($t = 0$). The incubation period consists, in this rough approximation, of two phases. Initially, there is a lag phase given by $(1/b)h_1$ followed by a growth phase of infected cells given by $(1/b)h_2$. Thus, the characteristic time scale of the incubation period is given by $1/b$, which denotes the average duration of the "infectious phase" of a cell.
2. Conversely, if $a << b$, we obtain $t \approx (1/a)(h_1 + h_2)$. Again there are the same two phases of infection dynamics, but this time the characteristic time scale is $1/a$, which denotes the average duration of the "latent phase" of an infected cell.

Case (1) may be more likely than case (2) because it implies that the spread of infectious prions in the brain is faster than the spread of cell death. This seems to be supported by experimental evidence.

Both approximations are under the assumption that $R_0 >> 1$. In both cases, we find that the characteristic time scale of the incubation period essentially reflects the lifetime of a prion infected cell. This is an important result. The character-

istic time scale of the disease should be determined by the intracellular dynamics. Note that the rate at which new cells become infected, βx, can only be time limiting if R_0 is (unrealistically) close to one.

However, there are two notes of caution necessary: 1) Eq. (3) neglects the complicated realities of spatial spread. If the rate of diffusion or transport of the prion particles is very slow in the brain of infected animals, then this process could dominate the overall dynamics. 2) Eq. (3) assumes direct cell-to-cell transmission of prion infection. If instead there is a long-lived extracellular reservoir of infectious prions (perhaps the amyloid placques), then it is possible that the lifetime of prion infected cells is short and that the time scale of the overall incubation period is determined by the lifetime of extracellular prion material.

Human and other prion diseases are characterized by a long incubation period (without many symptoms) and a sudden onset of disease with rapid progression to death. Can our models explain such a pattern of disease development?

The sudden onset of disease symptoms could be a consequence of a redundant organization of brain function. In certain regions of the brain, large numbers of neurons may die without causing symptoms. (In particular, if neuronal death occurs slowly over the time of many years, it is possible that other neurons could learn to compensate and prevent loss of function.) However, once a threshold is exceeded, the ability to compensate may be lost and symptoms may appear rapidly. This is then followed by a rapid destruction of any remaining function performed by this region of the central nervous system (CNS).

An alternative explanation for the sudden onset of clinical disease is provided by Payne and Krakauer[53] based on a more detailed model of the intracellular dynamics of PrP-res formation.

Another remarkable feature of prion diseases is the constancy of the incubation period in experimental infections. A single scrapie strain injected intracerebrally at high dose into inbred mice will generally give standard errors of less than 2% of the mean incubation period.[54] We suggest two main factors which may be responsible for the constancy of the incubation period: 1) unlike any other infectious agent, a prion strain (once established) cannot evolve during an individual infection; and 2) the immune response may not play an important role in prion infection dynamics. Therefore, the intrinsically stochastic nature of parasite mutation and selection during infection cannot generate heterogeneity in the rate of disease progression, and similarly, the great variability of the immune system also cannot provide its usual source of variation. It remains to be established, however, whether the amazing constancy of the incubation period is a characteristic feature of prion infections or whether viral or bacterial infection of CNS tissue would in the absence of immunity give rise to an equally constant pattern of disease progression in inbred animals.

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HOW DO PRION STRAINS "BREED TRUE"?

Perhaps the most puzzling observation of prion biology is the fact that different prion strains can be propagated indefinitely in strains of inbred mice which are homozygous for the PrP gene. In other words, different scrapie strains in the same mouse lead to different (but characteristic) incubation periods and to different (but characteristic) spatial distributions in the brain. This is surprising since it seems to imply—according to the prion hypothesis—that different PrP-res molecules can induce different conformational changes of the same PrP-sen molecule in such a way that each conformation of PrP-res generates an exact replica of its own conformation. Thus a given PrP-sen molecule has to exist in one healthy and several (up to 20) specific diseased conformations. Each of the diseased conformations must be able to interact with the healthy conformation in such a way as to reproduce its own conformation.

We propose two mechanisms by which different prion strains can breed true in the same animal.

Conformational Change Is a Consequence of Aggregation

If prion formation is indeed an ordered aggregation of PrP-sen molecules initiated by nucleation seeds which are small PrP-res aggregates, then it is conceivable that the molecular structure of the aggregate is dictated by the conformation of the nucleus.[25–27] The same PrP-sen monomers may be bound into different aggregates by different nucleation seeds (Fig. 4). Such an idea is also supported from studies of aggregation of other proteins. For example, supercoiling of the flagellar propeller is achieved by the coexistence of different conformations of flagellin in the filament. Different types of flagellar filaments can arise as a consequence of different nucleation seeds.[55] This mechanism can also explain why different prion strains can be propagated indefinitely in mice which are homozygous with regard to their PrP genes. The structure of the aggregate which forms the inoculum determines the conformation of the propagating prion. Protease digestion of different aggregates may generate different fragments even if they are derived from the same PrP-sen precursor.

This mechanism, strictly speaking, assumes that in a given animal there is one configuration of PrP-sen, which can be forced into different aggregates. The

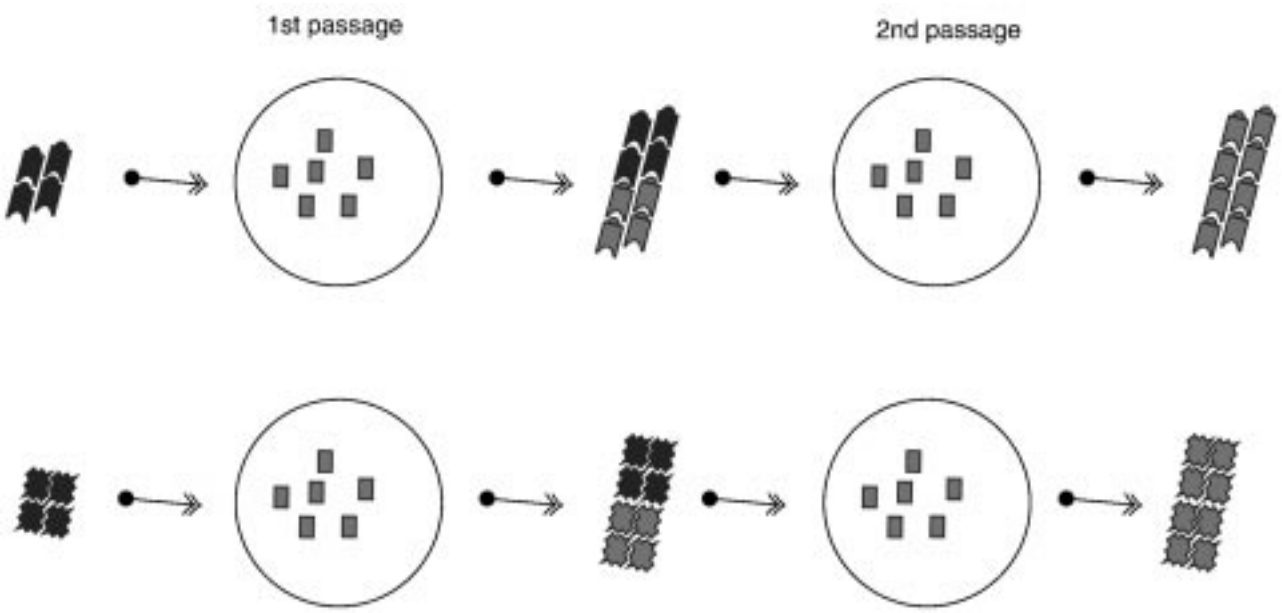

Figure 4. Generation of new strains and breeding true according to the nucleation-dependent mechanism. The same PrP-sen monomer is forced into different conformations which are determined by the structure of the nucleation seed. The figure shows how two different prion strains (from other species) are introduced into a group of inbred animals (large circles containing rectangles representing PrP-sen monomers). During the first passage, hetero-aggregates are being formed between the infecting prion protein and the host's own prion protein. The structure of the infecting prion is adopted as a consequence of extending the nucleation seeds. The infecting prion and the host prion are likely to have a different amino acid sequence. From the second passage onward the prion strains breed true; the structure of the original inoculum has been adopted and is carried forward by the host's own PrP protein. Strain characteristics (such as incubation period and lesion profile) should essentially settle after the second passage.

aggregates differ in their supermolecular structure and therefore also in the molecular conformation of their monomers. Aggregate conformation is solely determined by the structure of the nucleus that induces aggregation. While this mechanism can in principle explain how different scrapie strains can breed true in the same mouse, it does not explain naturally why different strains have well-characterized spatial distributions within the brain. Furthermore, we think that two or three different aggregation patterns may be realistic for the same protein monomer, but ten or more may be stretching the biological possibility of this mechanism.

Prion Isoform Selection Hypothesis

Experimental evidence suggests that there is only one PrP gene in a given animal and consequently all PrP proteins in this animal should have the same amino acid sequence. It is, however, conceivable that post-translational modification of the PrP protein (such as glycosylation) results in a variety of different specific PrP-sen molecules. The mature PrP protein has a molecular weight of about 33–35 kD, of which 7 kD (about 20%) consists of carbohydrates. A detailed carbohydrate structure analysis of PrP-res suggested that about 400 oligosaccharide combinations might exist for PrP-res.

Let us assume, therefore, that different isoforms of PrP-sen exist in the same animal and that these isoforms occur in specific spatial distributions in the brain. Subsets of neurons may synthesize specific isoforms of PrP-sen, each with the same amino acid sequence but different post-translational modifications. Prion induced conversion of PrP-sen into PrP-res would then only occur in those cells that produce an isoform of PrP-sen compatible with the PrP-res of the infecting prion.[56,57] According to such a mechanism it is sufficient to postulate that each isoform of the PrP protein can exist in one healthy configuration (PrP-sen) and one diseased configuration (PrP-res). It is no longer necessary to assume that a single PrP protein molecule can adopt several different diseased configurations.

It is also clear why strains breed true (Fig. 5). Suppose there are n isoforms of PrP-sen in a given animal, denoted A_1 to A_n. Assume that each isoform can be converted into a specific PrP-res conformation, denoted by A'_1 to A'_n. Each PrP-res isoform, A'_i, induces the conversion of its own specific PrP-sen isoform, A_i. Therefore, infecting an animal with A'_1 will induce conformational change of A_1 to produce more A'_1. Similarly, if an animal gets infected by A'_2 it will be induced to produce A'_2. And so on. Thus strains breed true.

Different isoforms of PrP-sen may be on specific subsets of neurons and therefore also in specific spatial distributions in the brain. Thus individual strains will accumulate in characteristic patterns during serial passage within inbred animals.

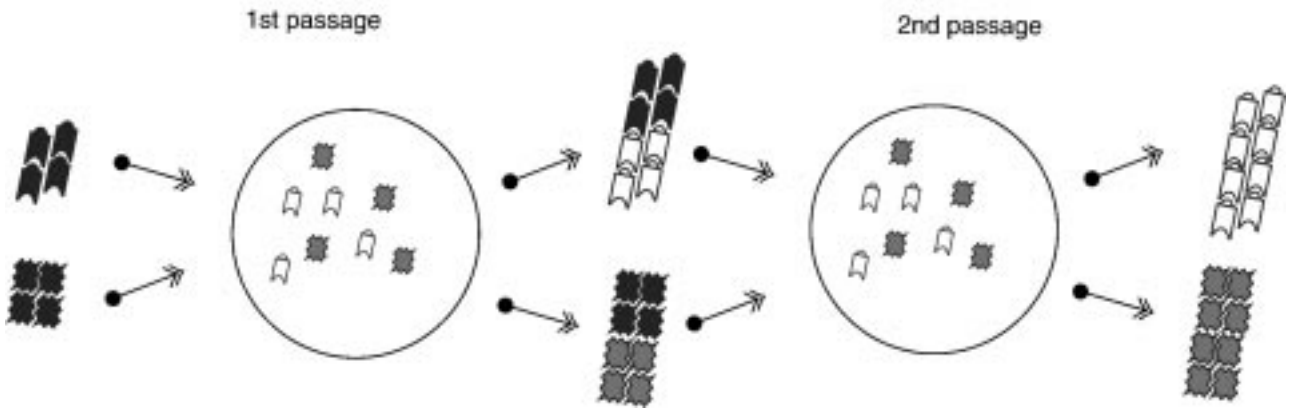

Figure 5. The prion isoform selection hypothesis suggests a different mechanism of how strains breed true. The assumption is that different isoforms of the PrP-sen molecule exist in an uninfected host. These isoforms have the same amino acid sequence but differ in post-translational modifications, e.g., in glycosylation residues. Different isoforms occur in specific subsets of neurons and/or have characteristic spatial distributions in the brain. Different PrP-res aggregates interact with specific PrP-sen isoforms. Individual PrP-sen isoforms can be converted into PrP-res aggregates which interact preferably with their own PrP-sen isoform. Therefore, strains breed true and have characteristic spatial distributions in the brain of infected animals. If prions from other species are introduced into a group of inbred animals, then the infecting prion may initiate the conformational conversion of one PrP-sen isoform into its own characteristic PrP-res structure. During subsequent serial passage, the PrP-res molecules again trigger conversion of their own specific PrP-sen isoform. Therefore, strains breed true and have a characteristic spatial distribution within the brain of inbred animals (given by the underlying spatial distribution of the specific PrP-sen isoform). The model can also be extended to allow for interaction among different isoforms.

If an animal gets infected with a prion from another species, then again it may be the case that this prion can interact with a specific A_i and initiate its conformational change into A'_i, which will then change more A_i into A'_i. In subsequent serial passages, the strain characteristics are defined by the properties of A'_i and will be conserved.

Finally, it seems likely that the interactions between the various isoforms of PrP-sen and PrP-res are *not* completely specific. It seems plausible that some isoforms may also induce conformational change of other isoforms. One can imagine a network of interactions between the A'_i and the A_i. In this case, a particular "prion strain" is not simply equivalent to a particular isoform, but to a subset of isoforms that can be triggered by each other.

ARE PRIONS THE MOST UNUSUAL INFECTIOUS AGENTS?

In this paper we presented several mathematical models and ideas on the replication dynamics of prions. If the prion hypothesis turns out to be correct, then prions represent a novel class of infectious agents with a fascinating mode of reproduction within hosts. Conclusive evidence, however, for the prion hypothesis is still lacking. The decisive experiment would be to synthesize the prion protein in a biochemical manner and demonstrate its ability to infect an animal. In the absence of this experiment, it cannot be ruled out that molecules other than the PrP protein (or indeed a virus) are involved in propagating infection.

We developed mathematical models for the aggregation-dependent mechanism of prion replication. The basic assumption is that conformational change of the healthy form, PrP-sen, into the diseased form, PrP-res, is a consequence of ordered aggregation. PrP-res aggregates grow by incorporating PrP-sen monomers, which then adopt PrP-res structure. PrP-res aggregates break and thereby form new nucleation seeds. Thus, fragmentation of PrP-res aggregates increases the number of aggregates, whereas aggregate growth increases the total amount of PrP-res. Both processes are necessary for prion replication under the aggregation-dependent mechanism.

The dynamics of such a process are characterized by a slow exponential increase of the total amount of PrP-res followed by saturation at an equilibrium abundance of PrP-res which is much higher than the abundance of PrP-sen in an uninfected cells. This is a consequence of a much smaller turnover rate of PrP-res compared to PrP-sen.

Ordered aggregation of proteins usually requires nucleation seeds. Therefore, we studied a model where aggregates below a critical size are unstable and disintegrate into monomers. In such a model, formation of nucleation seeds represents a kinetic bottleneck for prion replication. Spontaneous cases of prion disease, where prion replication starts without infection, have to overcome this bottleneck. If small amounts of PrP-res are continuously being generated by spontaneous conversion of PrP-sen, then it may take a long time until—by chance—sufficient aggregates above

the critical size have been produced to initiate the infection process. This model can therefore explain the typically long incubation period of spontaneous prion diseases. It also becomes clear why overexpression of the PrP protein increases the likelihood of spontaneous disease: a higher abundance of PrP-sen increases the rate of nucleus formation. Infection of an animal with prions may circumvent the slow kinetics of nucleus formation. Nucleation seeds are contained in the initial inoculum.

The length of the incubation period of transmitted prion disease may be explained by the slow exponential growth of PrP-res in any one infected cell. We showed that in the special case of prion infection, which essentially represents the spread of an infectious agent within a static host cell population (neurons do not divide), the incubation period is determined by the slowest stage in the life cycle of a prion. This is either the lifetime of an infected cell or the lifetime of infectious prion material outside of cells. In the first case, the lifetime of a prion infected cell would be comparable to the total length of the incubation period, i.e., months to years depending on the prion strain and the host species. It cannot be ruled out, however, that the lifetime of infected cells is shorter and that the main temporal component of the incubation period is a slow spatial spread (diffusion or transport) of the infectious agent.

The incubation period of experimentally infected mice is usually extremely constant (2% variation). We suggest that this is a consequence of 1) the fact that prions—unlike viruses or bacteria—cannot evolve during infection and 2) that immune responses may not affect the rate of prion propagation.

Finally, we suggested two mechanisms by which different prion strains can breed true in the same group of inbred animals. 1) According to the nucleation-dependent mechanism, the same PrP-sen molecule can be forced into different structures of PrP-res; PrP-res structure is completely determined by the structure of the nucleation seeds which initiate infection. 2) According to the prion isoform selection hypothesis, different isoforms of PrP-sen exist in an animal prior to infection. (These isoforms are caused by post-translational modification, e.g., specific glycosylation.) Incoming PrP-res nuclei can then initiate the conformational change of specific isoforms. Each PrP-res isoform subsequently converts its own specific PrP-sen isoform. Both mechanisms may operate. The prion isoform selection hypothesis also provides a natural explanation for the characteristic spatial distribution of lesion profiles caused by different prion strains in the same group of inbred mice.

. . . amyloid formation (or ordered aggregation of proteins) has been suggested as a crucial mechanism for several neurological disorders such as Alzheimer's disease, Parkinson's disease, Down's syndrome, and natural senescence.

The last word has not been spoken. We worked under the assumption that the prion hypothesis is correct. If a virus is to be found, then our mathematical models of prion propagation could become unnecessary. It should be noted, however, that amyloid formation (or ordered aggregation of proteins) has been suggested as a crucial mechanism for several neurlogical disorders such as Alzheimer's disease, Parkinson's disease, Down's syndrome, and natural senescence. Thus we cannot resist the temptation of believing that the mathematical models of this paper are of some wider significance.

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APPENDIX A

Here we analyze the prion propagation dynamics described by Eq. (1) with the assumption that aggregates break with equal probability at each site, $b_{i,j} = b$, and that all aggregates grow at a uniform rate, $\beta_i = \beta$. In this case we obtain

$$\dot{x} = \lambda - dx - \beta xy$$
$$\dot{y}_i = \beta x(y_{i-1} - y_i) - ay_i + 2b\sum_{j=1+1}^{\infty} y_j - b(i-1)y_i \quad i = 1,2.... \tag{A1}$$

Analytical insights into this model can be derived from the fortunate coincidence that Eq. (A1) can be closed by summation over all i. Summing y_i over all i, we obtain an equation in y and z, where $z = \sum_i iy_i$. Summing iy_i over all i, we obtain an equation in x, y, and z. Thus, the system is closed and can be described as a system of three differential equations:

$$\begin{aligned} \dot{x} &= \lambda - dx - \beta xy \\ \dot{y} &= bz - (a+b)y \\ \dot{z} &= \beta xy - az. \end{aligned} \tag{A2}$$

Here $z = \sum iy_i$ denotes the total number of PrP monomers incorporated into PrP-res aggregates, whereas $y = \sum y_i$ denotes the total number of PrP-res aggregates.

In the absence of a prion infection, the level of PrP-sen is at the equilibrium $x_0 = \lambda/d$. Once Prp-res is introduced into the system, it can spread provided each PrP-res aggregate gives rise to more than one new PrP-res aggregate before it is removed. In other words, the basic reproductive ratio, R_0, of the prion infection has to exceed one. For Eq. (A2) the basic reproductive ratio is given by

$$R_0 = \frac{\beta x_0 b}{a(a+b)}. \tag{A3}$$

If $R_0 < 1$, then the prion infection is not self-propagating. The PrP-sen monomer abundance will remain at its uninfected equilibrium value, x_0. If, on the other hand, $R_0 > 1$, then the infection will take place and the abundance of PrP-res will increase exponentially. Thus, $R_0 > 1$ is the condition for aggregate growth and fragmentation to be an infectious, self-perpetuating process.

The condition $R_0 > 1$ can also be formulated as a minimum rate of aggregate fragmentation which is necessary for prion replication. The rate at which PrP-res aggregates break has to fulfill $b > a^2/(\beta x_0 - a)$, in order for the prion infection to spread.

The initial growth phase can be understood by rewriting the equation for y as

$$\dot{y} = y(bs - a - b) \tag{A4}$$

where $s = z/y$ is the average size of PrP-res aggregates. From Eq. (A2) we obtain

$$\dot{s} = \beta x + bs(1 - s). \tag{A5}$$

For a reasonable choice of parameters, PrP-res growth is very slow, and initially x will remain at its preinfection equilibrium value, $x_0 = \lambda/d$. Thus, s will quickly increase to

$$\bar{s} = \frac{1}{2} + \sqrt{\frac{1}{4} + \frac{\beta x_0}{b}}. \tag{A6}$$

Therefore, the initial growth of total PrP-res abundance is approximately given by

$$y(t) \approx y(0)\exp\left[b(\bar{s} - 1) - a\right]t. \tag{A7}$$

For $\beta\lambda/(bd) >> 1$, we have $\bar{s} \approx \sqrt{\beta x_0 / b}$ and

$$y(t) \approx y(0)\exp(b\bar{s} - a)t. \tag{A8}$$

Note that $z(t)$ will grow with the same exponential growth rate as $y(t)$, simply because s remains constant during this phase.

Eventually, x will start to decline, the growth of y and z will slow down, and the system will converge to an equilibrium given by

$$x^* = \frac{a(a+b)}{\beta b}, \quad y^* = \frac{\lambda b}{a(a+b)} - \frac{d}{\beta}, \quad z^* = \frac{\lambda}{a} - \frac{d(a+b)}{\beta b}. \tag{A9}$$

At equilibrium, the average length of PrP-res aggregates is given by $s^* = 1 + (a/b)$.

APPENDIX B

Here we analyze a model of prion aggregation that includes a minimum nucleation seed. Let us assume that all aggregates below a critical size, n, are essentially unstable and rapidly fall into pieces which convert back to PrP-sen. Suppose this happens at rate u. We have

$$\dot{x} = \lambda - dx - \beta xy + u\sum_{i=1}^{n-1} iy_i$$
$$\dot{y}_i = \beta x(y_{i-1} - y_i) - (a + u_i)y_i + 2b\sum_{j=i+1}^{\infty} y_j - b(i-1)y_i. \tag{B1}$$

Here, $u_i = u$ if $i < n$, and $u_i = 0$ if $i > n$. This system cannot be closed by summation. We can, however, obtain the following approximation:

$$\begin{aligned} \dot{X} &= \lambda - dX - \beta X(Y_1 + Y_2) + uY_1 \\ \dot{Y}_1 &= bZ_1 - (a + b + u)Y_1 + 2b(n-1)Y_2 \\ \dot{Z}_1 &= \beta XY_1 - (a+u)Z_1 + bn(n-1)Y_2 \\ \dot{Y}_2 &= bZ_2 - \left[a + b(2n-1)\right]Y_2 \\ \dot{Z}_2 &= \beta XY_2 - aZ_2 - bn(n-1)Y_2. \end{aligned} \tag{B2}$$

Here $Y_1 = \sum_{i=1}^{n-1} y_i, Z_1 = \sum_{i=1}^{n-1} iy_i, Y_2 = \sum_{i=n}^{\infty} yi, Z_2 = \sum_{i=n}^{\infty} iy_i$ and X = x. System (B2) is only an approximation for system (B1), because for the summation of Y_1 and Y_2 we have omitted the term $\beta x y_{n-1}$, and for Z_1 and Z_2 we have omitted $n\beta x y_{n-1}$. This implies that in system (B2) the subcritical aggregate sizes, described by Y_1 and Z_1, do not generate the larger aggregates, described by Y_2 and Z_2. Nevertheless, (B2) is an excellent approximation for (B1) if the initial conditions already contain prion aggregates of sizes larger than the minimum nucleation seed.

There are two types of basic reproductive ratios in system (B2). For aggregates below the critical size we obtain

$$R_1 = \frac{\beta X_0 b}{(a+b+u)(a+u)}. \tag{B3}$$

For aggregates above the critical size we obtain

$$R_2 = \frac{\beta X_0 b}{(a+bn)\left[a+b(n-1)\right]}. \tag{B4}$$

Consider the interesting case when $R_1 < 1$ and $R_2 > 1$. Thus, small aggregates alone cannot propagate, but large aggregates can propagate and establish an infection.

More generally, when $R_2 > 1$ we can distinguish two cases. If $u > (n-1)b$, then $R_2 > R_1$, and populations or large aggregates, Y_2, can be maintained. In this event, populations of small aggregates, Y_1, are also being maintained (even though the Y_2 populations "outcompete" them), being "fed" by the Y_2 terms in the equations for Y_1 and Z_1. On the other hand, if $u < (n-1)b$, corresponding to relatively low disintegration rates of small aggregates, then $R_1 > R_2$; in this event, Y_1 "outcompetes" Y_2, and asymptotically we have only small aggregates.

If infection is initiated only with aggregates below the critical size, then Eq. (B2) describes how Y_1 and Z_1 decline over time. The question is whether during this decline the system manages to seed y_n and therefore initiate the infection. The probability of this happening depends on the abundance of PrP-sen prior to infection and on the initial dose of small aggregates. We can calculate how many nucleation seeds, y_n, will be produced from a given inoculum, $y_1(0)$. We obtain, to a good approximation,

$$y_n = y_1(0)\left[\frac{\beta x_0}{\beta x_0 + a + u + (b/2)(n-2)}\right]^{n-1}. \tag{B5}$$

One can alternatively ask, how long will it take until the prion replication process is initiated by formation of y_n, if there is a constant production rate, δ, of y_1. In this case, there are low level steady-state abundances for all y_i. If the steady state of y_n is larger than one, then y_n will be produced rapidly, at a time scale of

$$t \approx n\exp(-\beta x_0 - a - u). \tag{B6}$$

If the steady-state level of y_n is less than one, we find a characteristics time scale of

$$t \approx (1/\delta)\left[(\beta x_0 + a + u)/(\beta x_0)\right]^{n-1}. \tag{B7}$$

If $n >> 1$, then this represents a time scale much bigger than $1/\delta$.

If infection occurs with large aggregates then the system is similar to the simple model described in Appendix A. For the average size of PrP-res polymers above the critical size, $s = Z_2/Y_2$, we obtain from Eq. (B2)

$$\dot{s} = \beta x - bn(n-1) + bs(2n-1-s). \tag{B8}$$

For the initial phase of prion infection, where X remains roughly constant ($X \approx X_0$), s rises quickly to

$$\bar{s} = n - \frac{1}{2} + \sqrt{\frac{1}{4} + \frac{\beta X_0}{b}}. \tag{B9}$$

For $\beta X_0 >> b$ this is approximately

$$\bar{s} = n + \sqrt{\frac{\beta X_0}{b}}. \tag{B10}$$

The exponential growth rate of PrP-res aggregates above the critical size is now given by

$$Y_2(t) \approx Y_2(0)\exp\left[b(\bar{s} - 2n + 1) - a\right]. \tag{B11}$$

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