Species barrier in prion diseases: a kinetic interpretation based on the conformational adaptation of the prion protein

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Prion diseases are thought to result from the conformational change of the normal cellular prion protein to a pathogenic protease-resistant isoform. However, brain extracts not containing the protease-resistant isoform of the prion protein can be infectious following interspecies transmission. The 'protein-only' hypothesis of pathogenesis is extended to provide possible explanations which could be interpreted in terms of a different infectious agent. It is proposed that normal cellular protein (PrP^C) may be transformed into a form (PrP*) that is conformationally distinct from the host-specific abnormal isoform (PrP^{Sc}). In infection from a heterologous donor, the dimeric forms of heterologous PrP^{Sc}, which may catalyse the formation

of host PrP* from PrP^C, host PrP* and host PrP^{Sc} are all considered to be capable of catalysing, to some extent, the conversion of PrP^C into PrP^{Sc}. However, depending on the species involved, PrP* may, or may not, be pathogenic, and may, or may not, be sensitive to proteolysis. It is shown, by numerical integration of the differential rate equations derived from this model, that a strain may be stabilized after two or three passages through a different species and that transmission might occur in the absence of detectable protease-resistant prion protein. The natural transmission of scrapie to cattle is discussed in relation to the model.

INTRODUCTION

There is much biochemical and genetic evidence [1–8] that the agent responsible for transmissible spongiform encephalopathies (or prion diseases) is a pathogenic conformational isomer of a normal cellular protein (the prion protein PrP^C). The conformational change in the structure of the prion protein (PrP) is believed to be the determining event leading to these fatal, neurodegenerative disorders.

Recently, several theoretical studies have shown that the postulated mechanism for the propagation of prion diseases, in which the catalyst is an oligomeric assembly of the pathogenic isoform PrP^{Sc} of the protein, corresponds to a system with dynamic bistability and threshold properties [9–12]. It has two distinct and stable steady states, one of which is associated with disease, the other with health. Infection is thus a switch between these two alternative stable steady states. Exogenous PrP^{Sc} (or overproduced PrP^C) can push the system into the disease state. The dynamic component allows us to understand several features of prion diseases that static models cannot explain [13–15]. As an example, unrealistic values of rate parameters [16] would be needed to interpret sporadic diseases (occurring at random, without detectable cause) as the result of the rare spontaneous conversion of prion protein from the normal to the pathogenic isoform. In contrast, the dynamic approach shows that natural fluctuations of the turnover rate of the normal prion protein (or other parameters) can drive the system above the threshold and into disease onset.

One of the characteristics of the agent responsible of some prion diseases (mad cow disease, for example) is its great ability to infect other species, whereas other spongiform-encephalopathy agents are only readily transmitted within a single species. As a rule, the species barrier leads to significant prolongation of the incubation period following interspecies transmission. For instance, on transmission of bovine spongiform encephalopathy or

scrapie to mice the incubation periods are longer than subsequent mouse-to-mouse passages [17]. During serial passages, the incubation period shortens and stable pathological properties are acquired [18,19]. Although transgenic studies argue persuasively that the species barrier is due to differences in the PrP gene sequences between mammals, paradoxical results have recently been obtained in studies of experimental transmission of the bovine agent to mice [20]. In some cases, no PrP^{Sc} isoform of the prion protein is detected upon primary (and even secondary) infection of mice with bovine prions, although corresponding mice brain extracts remain infectious for subsequent passages. This was taken as indicating that the prion protein may not be the primary agent responsible for prion diseases and that the true agent is a nucleic acid.

In the present paper we show that a simple model based on the conformational adaptation of the prion protein to the new host is able to explain these paradoxical results. This 'protein-only' model gives a kinetic interpretation of the species barrier problem, lag characteristics observed in serial passages and failure to detect the PrP^{Sc} isoform in infectious brains. Moreover, counter-intuitive predictions of the model allow us to suggest a reliable protocol for ascertaining whether any particular prion disease is transmissible between species.

MODEL

An intermediary conformational state

Experimentally, species adaptation is studied by means of serial passages in a single host strain. The primary infection is the inoculation of host brains with homogenate prepared from a heterologous donor (first passage). Then, brain extracts obtained after the first passage are used to inoculate a new series of host animals of the same species. Generally, three passages are needed to stabilize the strain (i.e. no further decrease in the incubation period is observed for subsequent passages). This strongly

Abbreviations used: PrP, prion protein; PrP^C, cellular isoform of PrP; PrP^{Sc}, pathogenic isoform of PrP; PrP*, intermediary conformational state of PrP; *inocPrP^{Sc}*, inoculated PrP^{Sc} from a heterologous donor; *hostPrP^{Sc}*, stabilized PrP^{Sc} in the host species.

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Scheme 1 Conformational adaptation of the prion protein in interspecies transmission.

The pathogenic protein from the heterologous donor (*inocPrP^{Sc}*) catalyses the transformation [rate v_{i0} (' v_{i0} ')] of the normal, *PrP^C* host prion protein into an intermediary conformational state, *PrP**. *PrP** dimerizes [dissociation constant K_z (' K_z ')] and the dimer catalyses the conversion [rate v_{t1} (' v_{t1} ')] of *PrP^C* into the final, stabilized *PrP^{Sc}* isoform (*hostPrP^{Sc}*). *hostPrP^{Sc}* can also dimerize [dissociation constant K_y (' K_y ')] and the dimer contributes to the autocatalytic transformation [rate v_{t2} (' v_{t2} ')] of *PrP^C* into *hostPrP^{Sc}*. The spontaneous, uncatalysed conversion of *PrP^C* into *hostPrP^{Sc}* is assumed to be very slow [rate v_s (' v_s ')]'. All these reactions occur in an open system in which the normal *PrP^C* prion protein turns over [input and output rates v_i (' v_i ') and v_o (' v_o ') respectively]. The slow-leak terms v_{l0} (' v_{l0} ') and v_{l1} (' v_{l1} ') are associated with the monomeric forms of the *PrP** and *hostPrP^{Sc}* species respectively.

suggests that, during the course of the primary infection, at least one intermediary conformational state, *PrP**, is formed. The *PrP** protein may then catalyse the formation of the host-specific *PrP^{Sc}* isoform. This form converts the normal (*PrP^C*) protein into a likeness of itself and is thus autocatalytic. Studies of the species specificity of the conversion in a cell-free system have shown that the relative conversion efficiencies correlate with the relative transmissibility of the strain [21]. Hence, the species barrier seems related, at the molecular level, to the kinetic characteristics of the conversion reaction of the prion protein. Three catalysts may be present simultaneously: the heterologous *PrP^{Sc}* isoform found in the first inoculum (*inocPrP^{Sc}*), the *PrP** intermediary state and the final, stabilized *PrP^{Sc}* isoform (*hostPrP^{Sc}*). The balance between all these forms changes during serial passaging. Moreover, the cellular prion protein turns over. Hence, a complex kinetic scheme (Scheme 1) is associated with this simple molecular mechanism.

Kinetic scheme

For the sake of simplicity, we assume that only the dimeric forms W_d (*inocPrP^{Sc}* species), Z_d (*PrP** species) and Y_d (*PrP^{Sc}* species) are catalytically active. Moreover, the equilibrium for dimerization of the *PrP** and *PrP^{Sc}* isoforms is assumed to be rapid. Mass-conservation equations may be written as:

$$Y_T = Y_m + 2Y_d; Y_m^2/Y_d = K_y \quad (1a)$$

$$Z_T = Z_m + 2Z_d; Z_m^2/Z_d = K_z \quad (1b)$$

in which Z_m and Y_m represent the concentrations of the monomeric forms of the *PrP** and *PrP^{Sc}* species respectively. K_z and K_y

are the dissociation constants for the corresponding equilibrium. According to [21,22], the rate laws v_{i0} , v_{t1} and v_{t2} for the catalysed steps can be written thus:

$$v_{i0} = \frac{k_{c0}W_d \cdot X}{K_{m0} + X} \quad v_{t1} = \frac{k_{c1}Z_d X}{K_{m1} + X} \quad v_{t2} = \frac{k_{c2}Y_d X}{K_{m2} + X} \quad (2)$$

In these equations, X is the substrate concentration (*PrP* species). Note that the amount of pathogenic, heterologous protein inoculated at the first passage (W_d) is a constant. The rate of biosynthesis of *PrP* is taken as a zero-order rate equation ($v_i = \text{constant}$). All other individual reactions are assumed to correspond to first-order rate equations:

$$v_o = k_0 X \quad v_s = k_s X \quad v_{f0} = k_{f0} Z_m \quad v_{f1} = k_{f1} Y_m \quad (3)$$

The evolution of the concentrations of the species over time is described by the following differential equations:

$$\frac{dX}{dt} = v_i - X \left[\frac{k_{c0}W_d}{K_{m0} + X} + \frac{k_{c1}Z_d}{K_{m1} + X} + \frac{k_{c2}Y_d}{K_{m2} + X} + k_s + k_0 \right] \quad (4a)$$

$$\frac{dY_T}{dt} = X \left[\frac{k_{c1}Z_d}{K_{m1} + X} + \frac{k_{c2}Y_d}{K_{m2} + X} + k_s \right] - k_{f1} Y_m \quad (4b)$$

$$\frac{dZ_T}{dt} = \frac{k_{c0}W_d \cdot X}{K_{m0} + X} - k_{f0} Z_m \quad (4c)$$

Numerical solutions of differential equations (eqns. 4a–4c) can be calculated. Eqns. (1a) and (1b) have to be combined to give Z_m , Z_d , Y_m and Y_d as a function of the independent variables Z_T and Y_T :

$$Y_m = \frac{K_y}{4} [\sqrt{1 + 8Y_T/K_y} - 1]; \quad Y_d = \frac{Y_m^2}{K_y} = \frac{(Y_T - Y_m)}{2} \quad (5a)$$

$$Z_m = \frac{K_z}{4} [\sqrt{1 + 8Z_T/K_z} - 1]; \quad Z_d = \frac{Z_m^2}{K_z} = \frac{(Z_T - Z_m)}{2} \quad (5b)$$

Steady-state values

The steady states of the system (4a–4c) correspond to X^* , Y_T^* and Z_T^* values such that the conditions $dX/dt = 0$, $dY_T/dt = 0$ and $dZ_T/dt = 0$ are satisfied simultaneously. From eqn. (4c) and the additional condition $dZ_T/dt = 0$, Z_m and Z_d can be expressed as a function of X . Hence, the steady states of the system (4a–4c) can be calculated by solving the second-order autonomous system (eqns. 6a and 6b):

$$\frac{dX}{dt} = v_i - X \left[\frac{k_{c0}W_d}{K_{m0} + X} + \frac{k_{c1}\tilde{Z}_d}{K_{m1} + X} + \frac{k_{c2}Y_d}{K_{m2} + X} + k_s + k_0 \right] = 0 \quad (6a)$$

$$\frac{dY_T}{dt} = X \left[\frac{k_{c1}\tilde{Z}_d}{K_{m1} + X} + \frac{k_{c2}Y_d}{K_{m2} + X} + k_s \right] - k_{f1} Y_m = 0 \quad (6b)$$

Where

$$\tilde{Z}_d = \frac{Z_m^2}{K_z} = \frac{1}{K_z} \left[\frac{k_{c0} \cdot W_d \cdot X}{K_{m0} + X} \right]^2 \quad (7)$$

and Y_m and Y_d are given by eqn. (5a).

The solutions X^* and Y_T^* of eqns. (6a) and (6b) were calculated numerically, using a method based on the Levenberg–Marquardt algorithm [23]. Y_m^* and Y_d^* were calculated from eqn. (5a) (with $Y_T = Y_T^*$) and Z_m^* and Z_d^* from eqn. (7) (with $X = X^*$). Finally, Z_T^* was given by:

$$Z_T^* = Z_m^* + 2Z_d^* \quad (8)$$

These data allowed nullcline analysis to fix the number and the stability properties of the steady states of the system.

In all cases, inoculations at the second and subsequent passages are simulated by a pulse of 15 % of the steady-state concentration obtained for the various species at the end of the previous passage (dilution effect due to inoculation).

Unless stated otherwise, we chose for numerical simulations (standard values of parameters): $v_i = 10$, $k_{c0} = 1$, $K_{m0} = 1$, $k_{c1} = 0.2$, $K_{m1} = 1$, $k_{c2} = 1$, $K_{m2} = 1$, $k_s = 0.05$, $k_0 = 7.5$, $k_{f0} = 0.3$, $k_{f1} = 0.75$, $K_y = 1$, $K_z = 10$.

Properties of the intermediary conformational state

The PrP* intermediary conformational state may have distinctive kinetic and thermodynamic features different from those of the heterologous *inocPrP^{Sc}* protein and also from those of the stabilized PrP^{Sc} isoform. Nevertheless, these particular features are not necessarily related to the intrinsic pathogenicity or to the relative resistance to proteinase K of the intermediary isoform. Hence, observations have to be examined according to several possibilities: PrP* may be pathogenic or not, and sensitive or insensitive to proteolysis. Any combination may exist, depending both on the host and donor species.

RESULTS

Even in the simplest case, the pathological process does not behave as a dose-response system

In the limiting case where the PrP* isoform has the same catalytic and thermodynamic characteristics as the final conformational state, PrP^{Sc}, the dynamic process is similar to that previously described [9–11] for infection with a brain extract from a homologous donor: the system exhibits properties of bistability. Briefly, as long as the activity or the quantity of *inocPrP^{Sc}* in the inoculum does not exceed a threshold, the system returns to the initial, low-*hostPrP^{Sc}* steady state. In these conditions, foreign *inocPrP^{Sc}* protein is spontaneously eliminated. In contrast, when the activity or the quantity of *inocPrP^{Sc}* is above the threshold, the system jumps to the alternative, high-*hostPrP^{Sc}*, steady state. Bistability and associated threshold properties thus prevent the probability of cells or organisms developing the disease being in proportion to the dose of pathogenic inoculated protein. The disease thus differs from classical dose-response systems.

In this simple case, low-*hostPrP^{Sc}* and high-*hostPrP^{Sc}* steady states correspond to normal and pathogenic steady states respectively. No significant decrease in the length of the incubation period is to be expected from serial passages, because host PrP^C is presumably not a better substrate for conversion by *hostPrP^{Sc}* than for conversion by PrP*. This might correspond to very special cases in which homotopic relationships between the host and the donor are so close that there is no effective species barrier between the two species. This situation does not appear to be common: transmission of prions from one species to another is usually very inefficient, at least at the first passage.

Several serial passages stabilize the strain

When PrP* and *hostPrP^{Sc}* isoforms have distinctive kinetic and/or thermodynamic properties, new qualitative behaviours may be observed. Figures 1 and 2 present two different situations corresponding to distinct sets of parameter values. It shows how and why a strain may be stabilized at the second or the third passage.

In the situation shown in Figure 1, the species barrier exists, but its magnitude is small (low value of the K_z constant). At the

Figure 1 A strain may stabilize at the second passage

Inoculation of heterologous *inocPrP^{Sc}* protein at the first passage causes a transient increase in the concentration of the intermediary form of the protein, PrP* (broken line). This triggers the transition of the system from the normal low-*hostPrP^{Sc}* steady state to the pathogenic high-*hostPrP^{Sc}* steady state. At the second and subsequent passages, the amount of *hostPrP^{Sc}* contained in the inoculum is over the threshold and triggers the bistable transition. The standard values for the parameters were used, except for K_z which had a value of 5. This leads to the following species concentrations for the normal steady state: $X = 1.324$, $Y_f = 0.113$ and $Z_f = 0$. Hosts are assumed to be in this normal steady state before inoculation at zero time. For the first passage, we chose a W_d value of 10. Initial conditions for the second and third passages correspond to $X = 1.4243$, $Y_f = 43.91$, $Z_f = 0.585$ and $W_d = 1.5$ for the second inoculation and $X = 1.333$, $Y_f = 49.46$, $Z_f = 0.05$ and $W_d = 0.225$ for the third inoculation. Hence the second and third inoculations mainly consist of an addition of *hostPrP^{Sc}* species.

first passage, the concentration of dimeric PrP* species becomes transiently high enough to allow the transition between low-*hostPrP^{Sc}* and high-*hostPrP^{Sc}* steady states to occur. Thus *hostPrP^{Sc}* will be the main species found in extracts prepared for the second inoculation, which will start with a substantial complement of *hostPrP^{Sc}*. The incubation period, and thus the strain, will be stabilized at the second passage. Prolongation of the incubation period during the first passage corresponds to the time required for PrP* to reach the triggering concentration.

Imagine now that the association of PrP* into its oligomeric catalytically active form is thermodynamically unfavourable (high K_z value, Figure 2). Under these conditions, during the first passage, the dimeric PrP* form reaches a stationary concentration which is too low to jump the system towards the high-*hostPrP^{Sc}* steady state. Nevertheless, inoculated animals will develop the disease if the PrP* form is pathogenic. The inoculum used in the second passage mostly contains the monomeric, catalytically inactive, PrP* isoform and less of the corresponding dimeric, catalytically active, form. A lag period will be observed in the second passage corresponding to the time needed to produce significant amounts of *hostPrP^{Sc}*. However, this species easily forms catalytically active oligomeric structures, so that, during the second passage, conversion of PrP^C would mainly act, after the lag period, through catalysis by *hostPrP^{Sc}*. The catalytically active oligomeric form of *hostPrP^{Sc}* will constitute the majority species found in extracts prepared for the third inoculation. Hence, during the third and subsequent passages, the infection will start with a substantial complement of *hostPrP^{Sc}* and there will be no further change in the half-time of the conversion. Under these conditions the strain will be stabilized from the third passage onwards, as most frequently observed in experimental conditions.

Figure 2 A strain may stabilize at the third passage

Before any inoculation (at $t = 0$), hosts are assumed to be in the normal steady state ($X = 1.324$, $Y_T = 0.113$ and $Z_T = 0$) corresponding to the standard values for the parameters with $W_d = 0$. The first inoculation is associated with an increase in the $inocPrP^{Sc}$ concentration ($W_d = 40$). Inoculations at the second and third passages correspond to the following initial conditions: $X = 1.356$, $Y_T = 2.243$, $Z_T = 18.2$ and $W_d = 6$ for the second inoculation; and $X = 1.333$, $Y_T = 46.613$, $Z_T = 0.225$ and $W_d = 0.9$ for the third inoculation. The heterologous $inocPrP^{Sc}$ protein inoculated at the first passage increases the concentration of the intermediary form, PrP^* (A), which reaches a high stationary concentration. However, this concentration is not high enough to trigger the transition from the low- $hostPrP^{Sc}$ to the high- $hostPrP^{Sc}$ steady state (B). This occurs only at the second passage (essentially an addition of PrP^*), after a transient increase in the concentration of PrP^* . Inoculation at the third and subsequent passages corresponds to a pulse of $hostPrP^{Sc}$ that is sufficiently high to be above the threshold.

Several factors related to the process of conversion of the prion protein may influence the length of the incubation period. The steady-state concentration of the pathogenic species ($hostPrP^{Sc}$) is not very different between the second and third passages (Figure 2). In that case, the longer the half-time needed to reach this stationary concentration, the longer the predicted incubation period. Accordingly, the incubation period would be reduced between the second and the third passages, as expected. Estimation of the relative incubation period at the first passage is more difficult, when the PrP^* species is pathogenic. If an increase in the steady-state concentration of the pathogenic species decreases the incubation period, the latter would be

Figure 3 Variations of the half-time of the transition when the system is close to the threshold of bifurcation between low- and high- $hostPrP^{Sc}$ steady states

At the end of the first passage, the system reaches a low- $hostPrP^{Sc}$ steady state which is close to the unstable threshold. Under these conditions very slight variations in the v_f parameter (or any other parameter affecting the physiological state of the system) have disproportionately large effects on the half-time of the transition and thus on the incubation period of the disease. The standard values for the constant parameter were used, except for W_d ($= 29.8$) and k_0 ($= 0.5$). Initial conditions were as follows: $X = 0.1$, $Y_T = 30$ and $Z_T = 12$. The v_f parameter has the following values: 10.15 (curve 1), 10.116 (curve 2), 10.1152 (curve 3) and 10.1151 (curve 4).

actually prolonged during the first passage (corresponding to Figure 2), despite the PrP^* species appearing more quickly during this period than $hostPrP^{Sc}$ does during the second passage. However, a cascade of events (whose nature and kinetics are as yet unknown) might be required between the time that the concentration of the pathogenic prion isoform increases and the appearance of neurological dysfunctions. Moreover, the intrinsic pathogenicity of PrP^* and PrP^{Sc} may be different, making estimation of the relative incubation period difficult.

The existence of one intermediary isoform of the protein in the process of interspecies transmission of prion diseases allows interpretation of the stabilization of prion strains in new host species from the first, the second or the third passage. At least conceptually, the model could be readily developed to account for stabilization after a larger number of passages. For instance that each of the successive passages may mainly produce an intermediary isoform, which are progressively more similar to the final $hostPrP^{Sc}$ isoform. Nevertheless, such conceptual developments are not of great interest, as experimental data show that, in most cases, three passages suffice to stabilize the strain [18–20].

The disease can be transmitted in absence of detectable protease-resistant prion protein

In the situation described in Figure 2 the system does not jump towards the high- $hostPrP^{Sc}$ steady state at the first passage. Development of the disease at that time may be due to the accumulation of PrP^* , presumed to be pathogenic. If this isoform, like PrP^C , is highly sensitive to proteinase K treatment, the disease will develop at this passage, in the absence of a detectable proteinase-resistant isoform of the prion protein. Moreover, the disease will be transmitted at subsequent passages (Figure 2).

Figure 4 Possible variations of the steady-state properties of the host as a function of the amount of foreign *inocPrP^{Sc}* in the inoculum

Steady states, which are defined by constant values for the concentrations of all species, correspond to X , Y_T and Z_T such that eqns. (6a) and (6b) are satisfied simultaneously. The set of points which are the solutions of eqn. (6a) form a curve which is named the 'f-isocline'. Similarly, the solutions of eqn. (6b) form the second null isocline of the system ('g-isocline'). Hence, the steady states of system (6a–6b) are given by the intersection of the two null isoclines. An increase in the activity or the concentration of *inocPrP^{Sc}* [$W_d = 0$ (A), 2 (B), 10 (C), 20 (D) or 50 (E)] modifies the number and the nature of the steady states of the system. The system has successively two stable SS_1 and SS_3 steady states (A and B), one high-*hostPrP^{Sc}* SS_3 steady state (C and D) and, finally, one low-*hostPrP^{Sc}* SS_1 steady state (E). However, (F) shows that the low-*hostPrP^{Sc}* SS_1 steady state obtained in (E) is distinguishable from that observed in (A) or (B); under the conditions of curves (A) and (B) the SS_1 steady state is associated with a low level of PrP^* , whereas it is associated with a high-level PrP^* at high *inocPrP^{Sc}* (E). Hence if PrP^* is not pathogenic, there will be a window in the level of *inocPrP^{Sc}* which triggers the disease at the first passage. Curves were calculated with the standard values of the parameters, except for k_{f0} , which had a value of 0.5.

This situation typically corresponds to that described by Lasmézas et al. [20] in the case of the transmission of the bovine agent to mice.

Suppose now that the PrP^* isoform is not pathogenic but sensitive to proteinase K, like the cellular PrP^C prion protein. In the conditions shown in Figure 2 the accumulation of PrP^* at the

first passage will not lead to the development of the disease. However, this accumulation will induce the transition between low-*hostPrP^{Sc}* and high-*hostPrP^{Sc}* steady states at the second passage, i.e. the disease will develop at that time. This pattern was observed when mice lacking the PrP gene were inoculated with either the Chandler scrapie strain or the mouse-adapted

Fukuoka-1 strain of Creutzfeldt–Jakob disease [24,25]. Hence, conformational adaptation of the prion protein can also explain how a healthy donor, with no detectable proteinase-resistant isoform of the protein, is able to transmit a prion disease.

Slight variations in the physiological state of the host can induce a spread distribution of the incubation period before the strain is stabilized

Except in the case where the PrP* isoform is pathogenic, the disease results from the jump-like transition between the two alternate stable steady states of the system. The consequence of this transition is a sudden large increase in the concentration of the PrP^{Sc} isoform. When the normal, low-*hostPrP^{Sc}* steady state of the system is close to the threshold of the bistable transition, very slight fluctuations in the normal physiological state of the host may have large effects on the half time of the transition. Nevertheless, the final levels of PrP^{Sc} observed in the pathogenic state will not vary significantly. Hence, variations observed in the incubation period only depend, in that case, on those of the half-time of the transition. For instance, under the conditions presented in Figure 3, a difference of 0.35% in the rate of synthesis of the PrP^C normal isoform (v_i parameter) would more than double the half-time of the transition. Obviously, greater fluctuations in the v_i parameter are required to trigger the same quantitative effects when the system is distant from the threshold of transition. For subsequent passages, the amount of *hostPrP^{Sc}* in the inoculum is very much greater than the corresponding threshold value, so that the strain stabilizes and possible fluctuations in the physiological state of the hosts are of no consequence.

Hence this model allows us to interpret the widespread variations (about a factor of 3) in the incubation period observed experimentally between individual mice for the second passage, when the primary inoculation of the bovine agent had not led to detectable protease-resistant prion protein [20].

The dose–response protocol might be not adapted to assessing the efficiency of interspecies transmission of prion diseases

Intuitively, the larger the number of infectious units in the inoculum, the greater the probability of interspecies transmission of prion diseases. This view underpins the classical dose–response protocol used in studies on the transmissibility of infectivity. When the prion protein adapts to its new host with the formation of (at least) one intermediary conformational state, PrP*, the situation is much more complex and the intuitive prediction is not necessarily correct.

If the catalytic activity of PrP* is sufficiently high (but lower than that of the host-adapted prion species, *hostPrP^{Sc}*), a paradoxical behaviour may be observed at the first passage (Figure 4). In the absence of foreign *inocPrP^{Sc}* (Figure 4a), the system has three steady states. Two of them (SS₁ and SS₃) are stable and associated respectively with low and high stationary concentrations of the pathogenic isoform *hostPrP^{Sc}* of the prion protein. As usual, the intermediary steady state, SS₂, is the unstable threshold. Starting from the normal (low-*hostPrP^{Sc}*) SS₁ steady state (Figure 4a), inoculation of a small amount (or low total activity) of *inocPrP^{Sc}* does not produce the transition towards the pathogenic SS₃ steady state, because both stable steady states continue to co-exist (Figure 4b). However, as the activity or the quantity of *inocPrP^{Sc}* increases, phase plane analysis indicates that SS₁ and SS₂ steady states will converge and, finally, the difference will disappear. Only the pathogenic (high-*hostPrP^{Sc}*) SS₃ steady state will persist (Figures 4c and 4d) and thus the transition towards the pathogenic steady state necessarily occurs.

Once again, for higher activities or concentrations of the *inocPrP^{Sc}* protein in the inoculum, the situation becomes qualitatively distinct: the high-*hostPrP^{Sc}* SS₃ steady state disappears and is replaced by the low-*hostPrP^{Sc}* SS₁ steady state (Figure 4e).

The SS₁ steady state obtained at low *inocPrP^{Sc}* concentration (Figures 4a and 4b) has distinctive features, distinguishable from those of the SS₁ steady state at high *inocPrP^{Sc}* concentration (Figure 4e): the first state is associated with a low stationary concentration of PrP*; the second one with a high PrP* concentration (Figure 4f). Hence, inoculated animals in the low-*hostPrP^{Sc}* SS₁ steady state will develop the disease when PrP* is high, if the PrP* intermediary species is pathogenic. However, the consequences are very different if the PrP* intermediary species is not pathogenic. In these conditions, there is a window in the activity or concentration of *inocPrP^{Sc}* protein in the inoculum for which the host will become ill at the first passage. If the *inocPrP^{Sc}* protein level is either too high or too low, the inoculated host will not develop the disease. It could nevertheless transmit the infection in subsequent passages. Thus, to demonstrate that a prion agent cannot infect another species, it is not sufficient to show that hosts inoculated with a massive intracerebral injection of infected donor brain homogenate do not develop the disease, or present detectable protease-resistant forms of the prion protein at the first passage. At least two additional criteria must be satisfied: (1) hosts must not develop the disease in subsequent passages and (2) smaller doses of the primary inoculation do not lead to a qualitatively distinct situation in which hosts can develop the disease.

The second criterion is entirely counterintuitive and would lead to very unconventional results in standard dose–response protocols.

DISCUSSION

Prion diseases present features which are unusual and not readily explained by familiar ‘orthodox’ mechanisms. The presence of spongiform lesions and overt gliosis is clearly linked to that of the protease-resistant isoform of the prion protein [20]. The role of this isoform in the pathogenesis of cerebral damage has been repeatedly highlighted [26,27]. The requirement for normal prion protein in the development of disease and pathological lesions [24,25,28,29] is now generally accepted. Although the prion protein is clearly involved in the pathogenic process of spongiform encephalopathies, it is less evident whether the protein itself is the transmissible component of the infectious agent.

Until now, the existence of the species barrier and the observation that brain extracts can be infectious despite not containing detectable protease-resistant isoforms of the prion protein, have been considered to weaken the ‘protein-only’ hypothesis. Moreover, paradoxical results obtained in studies of mice expressing human and chimaeric *PrP* transgenes [30,31] were interpreted on the basis of an interaction between host prion protein and a putative species-specific protein X. Since the conformation of a protein can be influenced by complex interactions with many other cellular components, the possible involvement of another protein in the interspecies transmission of prion diseases cannot be excluded. However, we show here that reasonable hypotheses concerning the adaptability of the prion protein itself allow interpretation of the main features observed in interspecies transmission of these diseases. This demonstration is relevant, since experiments have so far failed to find any additional molecular entity required for disease. Moreover, conformational adaptability of the prion protein is well-established: experiments dealing with hamster-adapted mink

strains [32] and human prions in a mouse model [33] argue persuasively that prion diversity is encrypted in the conformation of PrP^{Sc}.

The dividing line between our hypothesis postulating the existence of an intermediary conformation of the prion protein and that suggesting the involvement of another component in the propagation of the disease may not be as sharp as it first appears. In both cases, an additional element is proposed. However, the putative intermediary prion isoform is an internal component of the system; thus it modifies the dynamic characteristics of the prion protein. In contrast, the putative unknown, additional component would be external to the dynamic system itself; hence, it would modulate the way in which the system expresses its dynamic characteristics without changing the nature of these characteristics. As an example, the possible existence of the species-specific protein X, supposed to bind to PrP^C prior its conversion into PrP^{Sc} [30,32], cannot explain why several passages are generally needed to stabilize a prion strain in a new host species. During each of the serial passages, the amount of available protein X would be the same. As a consequence, no decrease in the length of the incubation period would be expected. The progressive adaptation of the prion protein to its new host, as postulated in our mechanism, agrees with lag characteristics observed in serial passages. In several circumstances we modelled, steady-state properties were quite different in two successive passages because the intermediary PrP* isoform acts directly on the dynamics of the system. We supposed that the PrP* isoform has kinetic and thermodynamic characteristics distinct from those of heterologous inoculated PrP^{Sc} and also those of stabilized host PrP^{Sc}. This agrees with the observation that polymorphism of either PrP^C or PrP^{Sc} has large effects on the cell-free conversion efficiencies of sheep prion protein [34].

Our model predicts more phenomena than it was set up to reproduce. Under some particular conditions of interspecies transmission, although a given dose of prion protein in brain extracts from a heterologous donor can give the disease, a higher dose leaves the host healthy but infectious. This counterintuitive prediction of the model is not usually examined in classical protocols of dose-response experiments. It emphasizes that transmission of a 'protein-only' infection to other species shares some features with conventional viral diseases: an individual may be healthy but potentially able to transmit prion infection to another species, as latent viruses can be transmitted between healthy organisms within a species. Accordingly, elimination of public-health risks arising from prion diseases may not be possible simply by excluding high-titre tissues from the food chain. Biochemical and immunological tools have to be developed to characterize and measure all the intermediary molecular forms of the prion protein which may exist during the course of the adaptation of a prion strain from one species to another.

Does the present model apply to natural transmission of prion diseases between species? The current epizootic of mad cow disease is believed to have been initiated by scrapie infection of cattle via contaminated meat and bonemeal in feed supplements containing cattle (50%) and sheep (15%) offal [35]. As the effective exposure was extremely low, the epidemic was subsequently driven by the recycling of infected cattle material which by-passed the species barrier and served to passage the infection within cattle population. Our model interprets the efficiency of the recycling process, since healthy cattle might have contributed to the adaptation of scrapie to cows. Progressive adaptation of the prion protein during recycling appears to be an efficient

mechanism for transmission of infection to other species, even when the effective dose is not high enough to overcome the species barrier at the first passages.

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