Prion diseases and the 'protein only' hypothesis: a theoretical dynamic study

Michel LAURENT

Service d'Imagerie Cellulaire, URA 1116, Bât. 440, Université Paris-Sud, Centre d'Orsay, 91405 Orsay Cedex, France

In the 'protein only' hypothesis, prion diseases are thought to result from the conformational change of a normal isoform of a prion protein (PrP^{C}) to a protease-resistant, pathogenic form called PrP^{Sc}. This conversion rests on an autocatalytic process requiring the presence of pre-existing PrP^{Sc}. Theoretical kinetic analysis of the dynamic process, including the turnover of the normal prion protein, shows that the system exhibits bistability properties, indicating that the very slow accumulation of the abnormal form of the protein in the brain could in fact be the consequence and not the cause of the disorders. The cause would be a transition between two alternative steady states of the system. The presence of a small amount of the PrP^{Sc} protein in lymphocytes does not necessarily constitute any indication of a non-symptomatic but infectious pathogenic state. Moreover, infectious prion particles should not be seen as necessarily composed of the abnormal isoform of the protein, as usually stated. Particles containing only an excess of the normal form of the protein might also be pathogenic. Compounds that can act on the turnover rate of the normal PrP^{C} protein could be a therapeutic strategy against prion diseases.

INTRODUCTION

A wealth of evidence [1–6] supports the contention that the agent known as a prion, responsible for the transmissible spongiform encephalopathies such as scrapie in sheep and goat, and 'mad cow disease' or Creutzfeldt–Jacob disease in humans, is a protease-resistant, isomeric form (PrP^{Sc}) of a constitutive host protein (PrP^{C}). The modified, pathogenic form would multiply by converting the normal protein into a duplicate of itself. Additional evidence for such an unusual pathogenic post-translational process was obtained recently by Bessen et al. [7], who showed that incubation of PrP^{C} from uninfected cells with PrP^{Sc} derived from mouse brains infected with different prion strains converts PrP^{C} into PrP^{Sc}. Although PrP^{C} is necessary for the production of PrP^{Sc} and the development of disease in transgenic mice [6], the prion model is not universally accepted because of its weakness in interpreting strain variations [8]. In the 'protein only' hypothesis, differences in prion strains are thought to be mediated by stable variations in the three-dimensional structure of PrP^{Sc}. PrP^{C} and PrP^{Sc} are chemically indistinguishable but differ in their secondary structures and physicochemical properties [9–11]. In particular, PrP^{Sc} is resistant to proteinase K under conditions where PrP^{C} is readily cleaved [12,13]. Moreover, PrP^{Sc}, unlike PrP^{C}, gives rise to cerebral amyloid formation, a highly ordered protein aggregate characterized by its insolubility and fibrillar structure [14].

Two alternative mechanisms have been proposed for the conversion of PrP^{C} to PrP^{Sc} in prion strains. The 'nucleation model' [5,14–16] supposes that several PrP^{Sc} monomers polymerize to form a nucleus. Once the nucleus has been formed, further accretions occur through the binding of PrP^{C} to the polymer, a conformational rearrangement ($PrP^{C} \rightarrow PrP^{Sc}$) taking place to adjust the incoming molecule PrP^{C} to the template. The trapping of PrP^{C} would be an essentially irreversible aggregation process that would drive the bulk conversion reaction. The alternative model, known as the 'refolding model' [1], proposes that constitutive PrP^{C} monomers would be unfolded to some extent and subsequently refolded under the influence of the PrP^{Sc} molecule, which would behave, directly or indirectly, as a chaperone. As an example, Liautard suggested that prions could be misfolded molecular chaperones [17].

In this paper, I present a quantitative description of the prion invasion that takes into account both the turnover of the normal prion protein and the autocatalytic property of the $PrP^{C} \rightarrow PrP^{Sc}$ conversion step. The analysis uses a set of differential equations that were resolved by numerical integration. Kinetic analysis of the corresponding dynamic process provides insights into the occurrence of sporadic prion diseases and also into the characteristics of prion infection and possible new effective therapies.

EXPERIMENTAL

A generic kinetic scheme

Taking into account the turnover of the normal isoform of the prion protein, both the 'nucleation model' and the 'refolding' model correspond to the following generic scheme:

(1)

in which step 3 is a nonlinear process (see below). Output reactions (steps 2 and 4, which correspond to the degradation of native PrP^{C} and to the formation of aggregates respectively) are taken as first-order rate equations: $v_2 = k_2[PrP^{C}]$ and $v_4 = k_4[PrP^{Sc}]$. Step 1 (which corresponds to the synthesis of native PrP^{C}) is considered in the present analysis as a zero-order kinetic

Abbreviations used: PrP^{C}, normal isoform of prion protein; PrP^{Sc}, pathogenic isoform of prion protein.

process ($v_1 = k_1$) because the level of PrP messenger RNA was shown to be unchanged throughout the course of scrapie infection [1]. In the sequence above, steps 1 and 2 are both rapid because the PrP^C protein has a high turnover rate: half-times for PrPC synthesis and degradation were estimated to be 0.1 and 5 h respectively [1,3].

Rate law for the autocatalytic process

In the refolding model the PrP^{Sc} species directly retroactivates PrP^C conversion, whereas in the nucleation model the formation of the nucleus, which is the rate-determining step, is characterized by a lag time (below a critical concentration of monomers) followed by a sigmoidal progress curve [15]. The lag time is markedly dependent on protein concentration [14]. Hence both nucleation-dependent polymerization and retroactivated conversion behave kinetically as autocatalytic processes [13]. The fact that a nucleation process exhibits the kinetic behaviour of a true autocatalytic process, such as that corresponding to the refolding model, has been previously emphasized in the study of deoxyhaemoglobin S gelation [18]. However, none of these mechanisms has been expressed mathematically and only sparse kinetic data are yet available. Hence it seemed better to me to consider a phenomenological equation for expressing the kinetics of the autocatalytic process (step 3) rather than to try to derive this equation from a hypothetical molecular model.

Phenomenologically, the rate law of such an autocatalytic process (step 3) can be written as:

$$v_3 = [PrP^C]\frac{a(1+b[PrP^{Sc}]^n)}{1+c[PrP^{Sc}]^n} \quad (2)$$

in which n, a, b and c are constants. This equation is very similar to the Hill equation (which is typical of autocatalysis in enzyme kinetics, with the n parameter close to the Hill coefficient): the graphical representation of the function $v = f([PrP^{Sc}])$ is sigmoid with a non-zero value for $[PrP^{Sc}] = 0$. It adequately fits the kinetics corresponding to the feedback activation of the $PrP^C \rightarrow PrP^{Sc}$ transition without making any particular assumption about the molecular mechanism leading to such kinetic behaviour. Of course, the constants have no physical meaning because the model has no molecular foundation. However, simple inspection of eqn. (2) shows that some constraints exist for the choice of parameter values: a corresponds to the rate constant for the $PrP^C \rightarrow PrP^{Sc}$ conversion process in the absence of any PrP^{Sc} form. Because this process is supposed to occur very slowly in the absence of PrP^{Sc}, we have to choose $a < k_1$ and $a < k_2$. The PrP^{Sc} species behaves as an activator only when $b \not\geqslant c$. For a given a value, the larger the ratio b/c is, the bigger is the amplitude of the feedback activation phenomenon. For all calculations we arbitrarily choose $n = 4$, $a = 0.1$, $b = 2$ and $c = 0.05$. Within the limits indicated above, changes in any of a, b or c by at least one order of magnitude do not qualitatively change the dynamic behaviour of the system.

Analysis of the dynamic behaviour

The evolution of the concentrations of the species over time is described by the following differential equations (v_i corresponds to the rate of step i and PrP^{Sc} denotes the nucleus PrP^{Sc} species in the nucleation model):

$$\frac{d[PrP^C]}{dt} = v_1 - v_2 - v_3 \quad (3)$$

$$\frac{d[PrP^{Sc}]}{dt} = v_3 - v_4 \quad (4)$$

in which v_3 has a sigmoidal dependence on PrP^{Sc} concentration.

Eqns. (3) and (4) were integrated by the Gear method [19]. The local stability properties of each of the steady states can be analysed by determining whether infinitesimal perturbations in $[PrP^C]$ or $[PrP^{Sc}]$ away from the steady state decay or grow with time [20], as previously described for a different scheme [21].

RESULTS

Steady states, which are defined by constant values of all concentration species, correspond to $[PrP^C]$ and $[PrP^{Sc}]$ values such that the conditions $d[PrP^C]/dt = 0$ and $d[PrP^{Sc}]/dt = 0$ are satisfied simultaneously. In the phase plane whose coordinates denote PrP^C and PrP^{Sc} concentrations, the set of points that are the solutions of the equation $d[PrP^C]/dt = 0$ form a curve called the PrP^C null isocline. Similarly the solutions of the equation $d[PrP^{Sc}]/dt = 0$ form the second null isocline of the system (PrP^{Sc} null isocline). Hence the steady state of the system described in eqns. (3) and (4) is given by the intersection of the two null isoclines. Over a wide range of parameter values the PrP^{Sc} null isocline is sigmoid and the PrP^C null isocline is monotonic, showing either one or three intersections with the PrP^{Sc} null isocline (Figure 1). Accordingly the system has either one or three steady states, depending on the value of the turnover rate of the normal protein PrP^C (Figures 1b and 1c). The stability properties of each of the steady states depend on its location on the sigmoid null isocline. As shown in Figure 1(a), two steady states (SS_1 and SS_3) are stable whereas the intermediary state SS_2 is unstable when three of them co-exist.

Sporadic prion diseases might simply result from a decrease of the turnover rate of PrP^C below a certain threshold. Such a decrease provokes a jump-like transition between the SS_1 and SS_3 steady states (Figure 2). The consequence of this transition is a sudden, marked increase in the concentration of PrP^{Sc} (approx. 40-fold with the parameter values of Figure 2). Note that for a different set of kinetic parameters ($b = 200$, $k_1 = 800$, $k_2 = 60$ and other values of the parameters as in Figure 1), the jump in PrP^{Sc} concentration (a 700-fold increase in that case) and the opposite jump in PrP^C concentration are such that: (1) the PrP^C isoform is replaced nearly stoichiometrically by PrP^{Sc}; (2) the PrP^{Sc} concentration is near zero before the transition occurs. Such variations reproduce those observed between normal and scrapie-infected Syrian hamster brain [22]. Hence the spontaneous conversion of normal to pathogenic states should not be seen as the direct consequence of a very slow, continuous accumulation in the brain of PrP^{Sc}. On the contrary, an increase in one of the kinetic parameters (for example those involved in the turnover rate of PrP^C) beyond a threshold has a triggering effect on the dynamics of the system. It is the consequent change in the rate of synthesis or degradation of PrP^C, and not the spontaneous conversion of PrP^C and PrP^{Sc} [13], that seems, fortunately, to be a very rare event. Finally, there is no reason to believe that the concentration of the PrP^{Sc} isoform of the protein has necessarily to be zero in a non-pathogenic cell. It is sufficient that this concentration remains below a bifurcation threshold towards the pathogenic state.

How does scrapie infection promote conversion? Figure 3 shows the temporal response of three non-pathogenic cells to the addition of two successive, small concentrations $\Delta_1[PrP^{Sc}]$ and $\Delta_2[PrP^{Sc}]$ of PrP^{Sc}. Before the additions, all the cells are in a normal but slightly different physiological state (the turnover

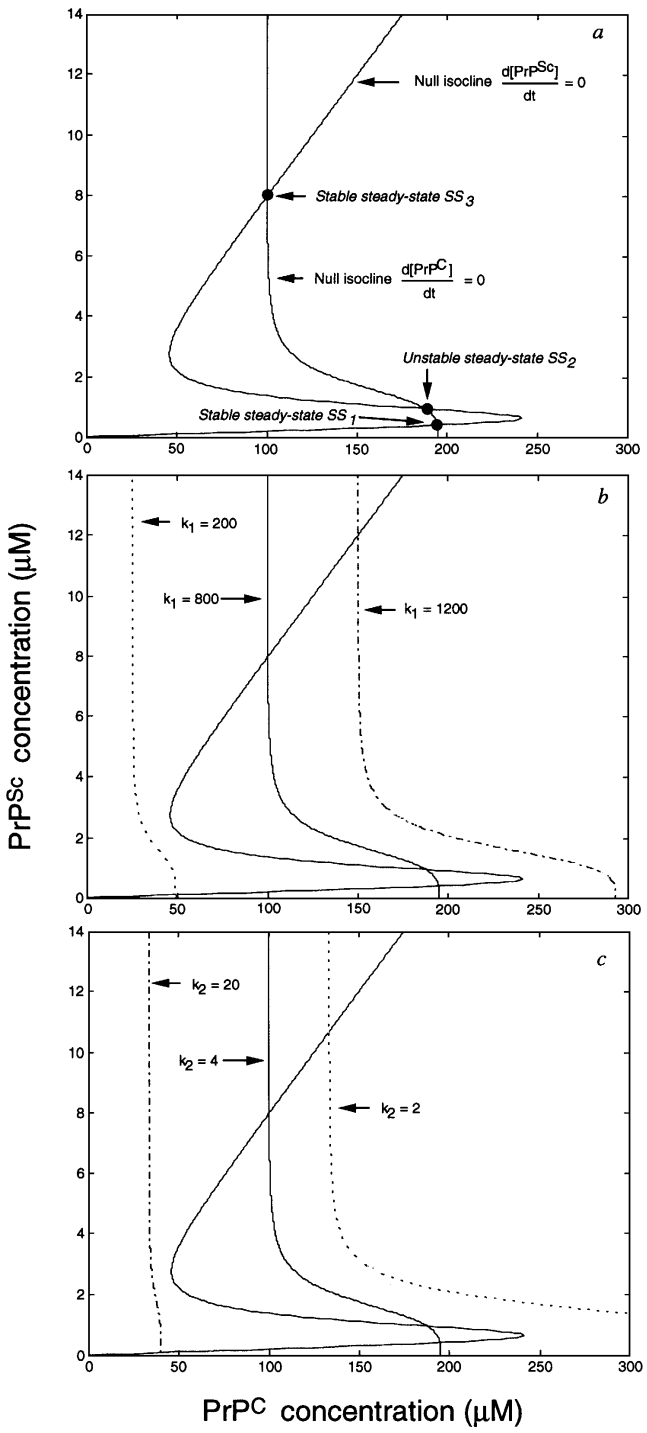

Figure 1 Stability analysis of the differential system

(**a**) Null isoclines and phase-plane portrait of the differential system, eqns. (3) and (4). The three steady states (solid circles) are located at the intersection of the two null isoclines (solid lines). Steady state SS_2, which lies in the region of negative slope of the sigmoid isocline, is unstable. In contrast SS_1 and SS_3 are stable and are associated respectively with low and high stationary concentrations of PrP^{Sc}. (**b**) Influence of the rate of PrP^C synthesis on the stability properties of the system. Increasing the rate constant k_1 modifies the position of the null isocline $d[PrP^C]/dt = 0$ but does not change the shape or position of the sigmoidal null isocline $d[PrP^{Sc}]/dt = 0$. The system has successively one globally stable, non-pathogenic SS_1 steady state (for $k_1 = 200$ μM/s), two locally stable SS_1 and SS_3 and one unstable SS_2 steady states (for $k_1 = 800$ μM/s) and finally one globally stable, pathogenic SS_3 steady state (for $k_1 = 1200$ μM/s). (**c**) Influence of the rate of PrP^C degradation on the stability properties of the system. A decrease in the rate constant k_2 from 20 to 2 s^{-1} leads to the same dynamic behaviour as that described in (**b**) for an increase in k_1. The rate constants were: (**a**) $k_1 = 800$ μM/s and $k_2 = 4$ s^{-1}, (**b**) $k_2 = 4$ s^{-1}, (**c**) $k_1 = 800$ μM/s; $k_4 = 50$ s^{-1} in all cases.

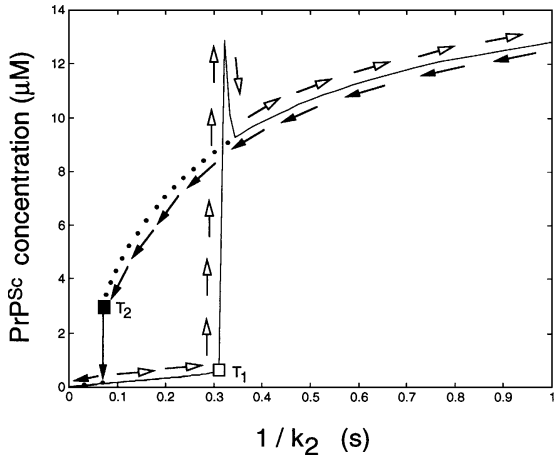

Figure 2 Influence of the turnover rate of PrP^C on the steady-state properties of the system described in eqns. (3) and (4)

Suppose that the system is initially in steady state SS_1 and that a continuous decrease in the rate k_2 of PrP^C degradation occurs (open arrows). Until the stationary state lies on the lower branch of the PrP^{Sc} null isocline, the PrP^{Sc} concentration is slightly readjusted in accordance with the solution trajectory. However, when the variable parameter becomes less than a threshold value (T_1, corresponding to the change in the sign of the slope of the PrP^{Sc} null isocline), the system moves to the upper branch of the PrP^{Sc} null isocline (SS_3 domain of stability) and a strong, sudden increase appears for the steady-state concentration of PrP^{Sc}. If parameter k_2 is now increasing (filled arrows) while the system is on the higher branch of stability of the PrP^{Sc} null isocline (steady state SS_3), the system moves down this branch until the slope of the null isocline turns negative (T_2 threshold). Then a jump-like transition occurs towards the lower branch of the PrP^{Sc} null isocline. The values of k_2 for which transitions between the alternative steady states occur are different depending on whether this parameter increases or decreases. Such transitions are called hysteretic [20]. The same dynamic behaviour is observed when the rate constant k_1 of PrP^C synthesis (step 1) is chosen as the variable parameter. The parameter values are as in Figure 1a.

rate of PrP^C is supposed to differ slightly from one cell to another). As a consequence, their threshold conditions of excitation are distinct. As long as the pulse of PrP^{Sc} concentration does not exceed the threshold, the system quickly returns to the initial, non-pathogenic steady state (Δ_1 perturbation in Figure 3b). In contrast, when the amplitude of the pulse is sufficiently high to pass over the threshold (Δ_2 perturbation in Figure 3b or Δ_1 perturbation in Figure 3c), a large amplification of the pulse occurs and the system jumps to the corresponding pathogenic, high-[PrP^{Sc}] steady state. However, when the turnover rate of PrP^C is so high that only a single, non-pathogenic low-[PrP^{Sc}] steady state exists, any addition of the PrP^{Sc} pathogenic form of the protein is spontaneously eliminated (Figure 3a), i.e. the cell cannot be infected. It is noteworthy that the length of the period during which the perturbation is applied has no influence on the possible occurrence of the transition. This possibility only depends of the nature of the perturbation below or above the threshold. Once the addition of PrP^{Sc} above the threshold is applied, the marked increase in the stationary PrP^{Sc} concentration is immediate (at least in this oversimplified model; see the Discussion section). Of course this change accelerates the rate of amyloid formation, but this process is not necessarily as rapid (it also depends on the kinetic parameters of aggregation).

As usual in multistable systems, perturbations of any of the variables are able to induce the transition between alternative steady states. This is shown in Figure 4 in which successive increments of PrP^C are supposed to be added to a cell that is initially in a normal state. The transition from the normal (low-[PrP^{Sc}]) to the pathogenic (high-[PrP^{Sc}]) state is obtained for the

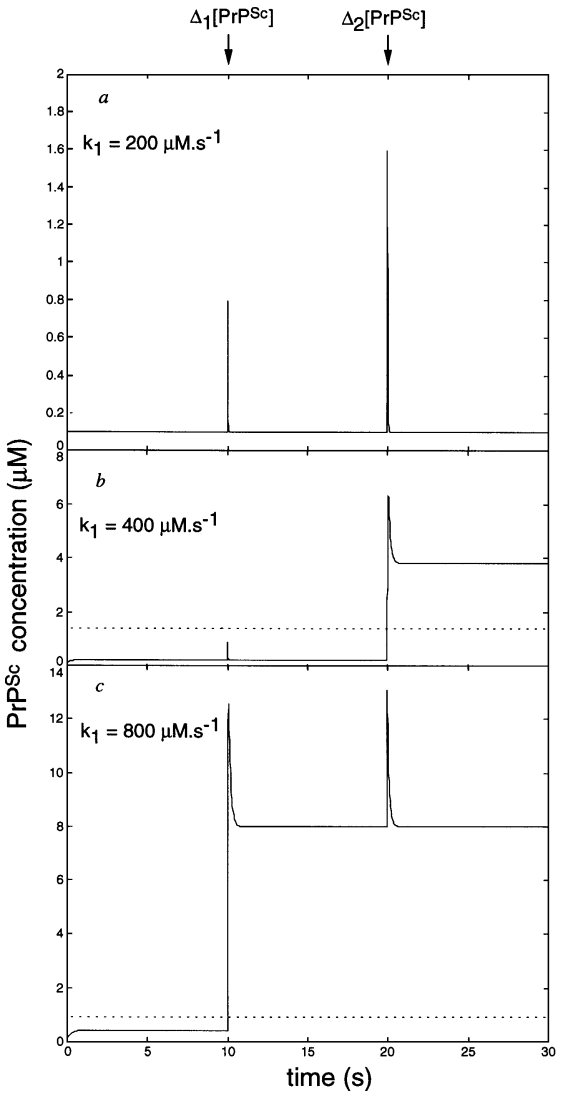

Figure 3 Evolution of PrP^{Sc} concentration with time under different conditions of infection by PrP^{Sc} ($\Delta_1[PrP^{Sc}] = 0.7\ \mu M$ and $\Delta_2[PrP^{Sc}] = 1.5\ \mu M$ excitations) for three non-pathogenic cells (a, b and c) that differ slightly in the value of the kinetic constant k_1 for PrP^C synthesis

Initially the stationary state of the three cells lies on the lower branch of stability (but at distinct positions) of the sigmoid PrP^{Sc} null isocline. In each case the dotted line denotes the $[PrP^{Sc}]$ threshold of excitation. In (**a**) none of the excitations is amplified and able to produce a transition between a low and a high $[PrP^{Sc}]$ steady state because only the low, globally stable $[PrP^{Sc}]$ steady state exists in that case (see Figure 1b). Hence the host cell cannot be infected. For a cell that has a slightly higher rate of PrP^C synthesis (**b**), the $\Delta_1[PrP^{Sc}]$ perturbation, below the threshold, is eliminated whereas the second $\Delta_2[PrP^{Sc}]$ pulse, beyond the threshold, causes a switch from the low to the high $[PrP^{Sc}]$ steady state, i.e. the host cell becomes infected. For the third cell, which has a still higher rate of PrP^C synthesis (**c**), the first excitation $\Delta_1[PrP^{Sc}]$ is sufficient to generate the transition to the pathogenic steady state. The second excitation $\Delta_2[PrP^{Sc}]$ has no additional effect in this case. Note that the transition from a normal (SS_1) to a pathogenic (SS_3) steady state corresponds to a large amplification (with the presence of an overshoot) of the pulse of excitation. The same behaviour would be observed if the rate constant k_2 were taken as the variable parameter. Rate constant k_1 was (**a**) 200, (**b**) 400 or (**c**) 800 μM/s; other parameter values were as in Figure 1(a).

third addition of PrP^C, the two first ones being below the threshold of excitation. Such behaviour would have to be tested experimentally, because the correct position and form of the sigmoid null isocline in a true experimental phase plane are as yet

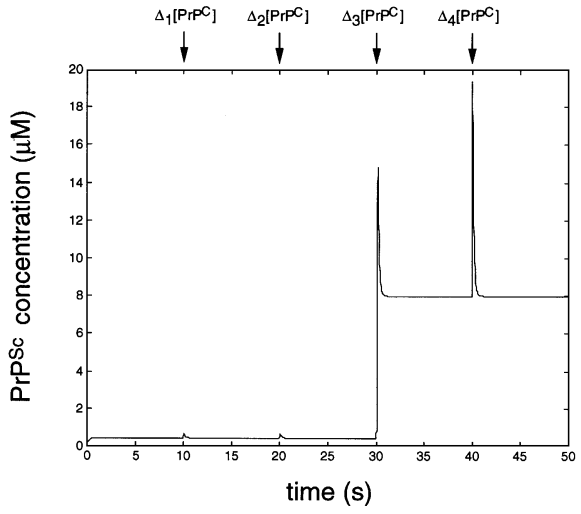

Figure 4 Evolution of the PrP^{Sc} concentration with time in an initially non-pathogenic cell, under different conditions of perturbation of the dynamics by PrP^C

The two first pulses of PrP^C ($\Delta_1[PrP^C] = \Delta_2[PrP^C] = 70\ \mu M$) do not exceed the $[PrP^C]$ threshold. Hence the fluctuation is spontaneously eliminated and the system comes back to its initial, low-$[PrP^{Sc}]$ steady state. In contrast, the third perturbation beyond the threshold ($\Delta_3[PrP^C] = 80\ \mu M$) provokes the transition from the initial, low-$[PrP^{Sc}]$ steady state to the pathogenic, high-$[PrP^{Sc}]$ steady state, i.e. the cell switches to the infected state. Note that after this third $[PrP^C]$ pulse the new stationary PrP^C concentration becomes lower that the initial one (see the shape of the null isoclines in Figure 1a). This observation also holds in the case of the $[PrP^{Sc}]$ perturbations (Figure 3). The last excitation ($\Delta_4[PrP^C] = 80\ \mu M$) does not produce any additional effect because the system is already in the high-$[PrP^{Sc}]$ steady state, i.e. this fluctuation is eliminated but the system remains in its pathogenic state. The parameter values are as in Figure 1(a).

unknown (i.e. the order of magnitude of the $[PrP^C]$ threshold is not known).

DISCUSSION

A model for prion invasion

A kinetic analysis of the dynamics of prion diseases shows that the corresponding metabolic system exhibits properties of bi-stability. Under controlled conditions the addition of quantities of PrP^{Sc} or, rather paradoxically, of the normal PrP^C protein, beyond a threshold triggers the transition to the pathogenic steady state. Hence the characteristics of prion infection differ from those of virus replication, healthy organisms being able to eliminate spontaneously amounts of foreign or endogenous PrP^{Sc} protein below the threshold. These results agree rather nicely with the observation that transgenic mice that exhibit an increase in PrP^C synthesis do not always become ill [1]: in the present model, as long as the PrP^C concentration does not exceed a threshold, the modification does not induce the disease.

The very low accumulation in the brain of PrP^{Sc}, as observed in sporadic prion diseases, might be only the consequence and not the cause of the disorders: a decrease in the turnover rate of the normal protein might be the primary event that switches the system into the pathogenic state. We may suppose that some point mutations in the *prnp* gene responsible for the synthesis of PrP^C would increase the frequency of this pathogenic event in producing an altered PrP^C protein whose turnover rate would be modified.

Finally, it is not surprising to observe that the presence of a small amount of the PrP^{Sc} isoform in lymphocytes is prevalent in

the human population [23]: this does not constitute any indication for a non-symptomatic but infectious pathogenic state. In a healthy organism, the concentration of the PrP^{Sc} is below a threshold value but is not necessarily zero. Obviously the limits of the accuracy of these conclusions are those of the 'protein only' hypothesis. Within these limits, the research on compounds that inhibit addition of monomer to the nucleus [14] can be seen as a possible therapy against amyloid formation. However, the best strategy would be to prevent the system from entering – or to allow it to leave – the dynamic zone in which the transition between normal and pathogenic steady states is possible. Hence compounds that increase the turnover rate of PrP^{C} would be promising in the research into effective therapies for prion diseases, both in prevention and cure.

On the observation of long incubation times in prion diseases

The present model shows that a change in PrP^{Sc} concentration beyond a threshold induces an immediate increase in the concentration of PrP^{Sc}. This seems to contradict the observation of incubation times up to several years in humans, and many hundred days in mice. In fact, incubation times are simply defined as the number of days from inoculation to onset of neurological dysfunction. A cascade of events (whose nature and kinetics are as yet unknown) might be required between the time that the concentration of PrP^{Sc} is increased and the time that the neurological dysfunction is detected. Moreover the kinetics of the transformation $PrP^{C} \rightarrow PrP^{Sc}$ could be determined in part by the efficiency and rate of association between the PrP^{C} and PrP^{Sc} isoforms, because the heterodimer $PrP^{C}-PrP^{Sc}$ could act as a catalyst for the conversion [24]. In such a case the bistable properties of the system would not be changed qualitatively but the transition between the alternative steady states might occur slowly [25].

The missing link?

From a fundamental point of view, this apparently paradoxical result on the ability for the PrP^{C} to induce prion diseases indicates that the molecular and dynamic approaches are complementary. Molecular data (particularly their kinetic components) must be available before dynamic studies can be performed. Dynamics permit the description of the global behaviour of the system as a result of the interactions between its components. However, the global properties of the system are not the sum of the properties of the components. New macroscopic properties stem from the existence of these interactions: basically, it is neither the high turnover rate of PrP^{C} nor the autocatalytic nature of the transformation of PrP^{C} to PrP^{Sc} that endows the system with properties of bistability. These properties result from the fact that PrP^{C}, which has a high turnover rate, is also involved in a branched, autocatalytic transformation leading to PrP^{Sc}.

From a molecular point of view, the occurrence of prion diseases relies on a mechanism in which a protein passes on its structural information to another protein molecule without any direct involvement of the genomic material. One question is whether this constitutes a very unusual pathogenic mechanism that is specifically linked to this particular protein or, alternatively, whether this mechanism might also enable other normal proteins to achieve and pass on their functional conformation. Such a hypothesis might constitute the missing link for the understanding, at the molecular level, of structural inheritance phenomena observed at the cellular level, especially in ciliates [26,27].

Received 18 January 1996/30 April 1996; accepted 14 May 1996

I thank Professor S. B. Prusiner for helpful comments, and Dr. G. Johannin and Dr. A. Fleury for careful reading of the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (URA 1116) and the Université Paris-Sud.

REFERENCES

1 Prusiner, S. B. (1991) Science **252**, 1515–1522
2 Weissmann, C. (1991) Nature (London) **349**, 569–571
3 Borchelt, D. R., Scott, M., Taraboulos, A., Stahl, N. and Prusiner, S. B. (1990) J. Cell Biol. **110**, 743–752
4 Caughey, B. and Raymond, G. J. (1991) J. Biol. Chem. **266**, 18217–18223
5 Kocisko, D. A., Come, J. H., Priola, S. A., Chesebro, B., Raymond, G. J., Lansbury, P. T. and Caughey, B. (1994) Nature (London) **370**, 471–474
6 Büeler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M. and Weissmann, C. (1993) Cell **73**, 1339–1347
7 Bessen, R. A., Kocisko, D. A., Raymond, G. J., Nandan, S., Lansbury, P. T. and Caughey, B. (1995) Nature (London) **375**, 698–700
8 Hope, J. (1996) Nature (London) **378**, 761–762
9 Stahl, N., Baldwin, M. A., Teplow, D. B., Hood, L., Gibson, B. W., Burlingame, A. L. and Prusiner, S. B. (1993) Biochemistry **32**, 1991–2002
10 Pan, K. M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J., Cohen, F. E. and Prusiner, S. B. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 10962–10966
11 Cohen, F. E., Pan, K. M., Huang, Z., Baldwin, M., Fletterick, R. J. and Prusiner, S. B. (1994) Science **264**, 530–531
12 Meyer, R. K., McKinley, M. P., Bowman, K. A., Braunfeld, M. B., Barry, R. A. and Prusiner, S. B. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2310–2314
13 Weissmann, C. (1995) Nature (London) **375**, 628–629
14 Jarret, J. T. and Lansbury, P. T. (1993) Cell **73**, 1055–1058
15 Gajdusek, D. C. (1988) J. Neuroimmunol. **20**, 95–110
16 Kocisko, D. A., Priola, S. A., Raymond, G. J., Chesebro, B., Lansbury, P. T. and Caughey, B. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 3923–3927
17 Liautard, J. P. (1991) FEBS Lett. **294**, 155–157
18 Hofrichter, J., Ross, P. D. and Eaton, W. A. (1974) Proc. Natl. Acad. Sci. U.S.A. **71**, 4864–4868
19 Garfinkel, D. and Marbach, C. (1977) Annu. Rev. Biophys. Bioeng. **6**, 525–542
20 Nicolis, G. and Prigogine, I. (1977) Self-Organization in Non-Equilibrium Systems, Wiley, New York
21 Laurent, M. and Fleury, A. (1993) FEBS Lett. **336**, 1–7
22 Prusiner, S. B., Scott, M., Foster, D., Pan, K. M., Groth, D., Mirenda, C., Torchia, M., Yang, S. L., Serban, D., Carlson, G. A., Hoppe, P. C., Westaway, D. and DeArmond, S. J. (1990) Cell **63**, 673–686
23 Manuelidis, E. E. and Manuelidis, L. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 7724–7728
24 Come, J. H., Fraser, P. E. and Lansbury, P. T. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 5959–5963
25 Demongeot, J. and Seydoux, F. (1979) in Élaboration et Justification des Modèles. Applications en Biologie (Delattre, P. and Thellier, M., eds,), pp. 519–536, Masson, Paris, France
26 Beisson, J. and Sonneborn, T. M. (1965) Proc. Natl. Acad. Sci. U.S.A. **53**, 275–282
27 Fleury, A. and Laurent, M. (1994) J. Eukaryotic Microbiol. **41**, 276–291