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Prions and their biophysical background

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1. Introduction

An extraordinary subject of basic research was being intensively studied long before its economic and medical impact became evident. In the early eighteenth century nobody had thought about BSE; nobody had predicted it. Nevertheless, there was a group of scientists who suggested that a long-recognised disease of sheep was not a normal disease but represented a dogma-violating principle of biology. The agent is not a virus but a prion.

What are prion diseases? The scrapie disease of sheep has been known for several hundred years; the phenomenology had already been well described in a veterinary textbook by Leopoldt in 1759 [1]. In this century scrapie was a limited agricultural problem in Scotland and Iceland. Some research was carried out continuously over many years, but up to the 1960s the disease remained an enigma. The disease, which seemed to be restricted to sheep and goats, was a lethal neurodegenerative disease with a low incidence and infection rate, with an incubation time amounting to several years. The agent was unknown, commonly regarded as an unidentified virus.

Interest increased drastically when suggestions were made that the enigmatic sheep disease and some human diseases might be related. In the 1920s the German neurologists Creutzfeldt and Jakob had already described a lethal disorder of the central nervous system, since called "Creutzfeldt–Jakob disease" (CJD). The disease started with uncorrelated movement and led to total mental disorder and death within about a year. The disease is very rare but occurs worldwide. More like an epidemic, a disease was observed of the primitive people in Papua New Guinea. The American physician Gajdusek discovered that this disease, called Kuru, was transmitted by ritual cannibalism, although it was not shown whether the brain material of the bodies was eaten or only brought into contact with the skin [2]. As a very peculiar feature, Gajdusek described the incubation time as being from a few years to decades, and therefore called the agent a "slow virus", without, however, having seen a virus.

Those diseases had been described completely independently for humans and animals. The link between them came from histopathology. In all cases spongiform deterioration of the grey matter was observed, and this observation suggested a related etiology [3]. Indeed, the transmissibility and the spongiform deterioration led to the name "transmissible spongiform encephalopathy".

2. The prion hypothesis

As early as 1966, Tikwar Alper suggested from the anomalous resistance of the scrapie agent against

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ionizing irradiation, that the target size is too small for a viral genome and that it behaves more like a protein of the order of 100 KDa [4]. This would mean that viruses are not the smallest agents known, but that particles smaller than viruses — so-called subviral agents — might exist. Other features became known pointing to subviral agents. For example, no viruses or virus-like particles could be identified in electron micrographs of highly infectious material; the simplest explanation was that the agent was too small to be detectable. As a further untypical feature of a virus, it was realized that the scrapie infection did not initiate an immune response. Studies on the stability of the agent were carried out by several research groups with a large variety of chemical, biochemical, and physical treatments. In general, the scrapie agent exhibited a high resistance to treatments which deactivate viruses.

It was Stanley Prusiner from the University of California in San Francisco who extended these studies systematically and summarized his own and others' results as follows:

(i) Chemical and physical procedures which modify or destroy nucleic acids (meaning also those of viruses, bacteria, fungi, etc.) do not deactivate the scrapie agent.

(ii) Chemical and physical procedures which modify or destroy proteins, do, however, deactivate the scrapie agent.

Nobody except Prusiner was brave enough at that time to formulate the logical conclusion from the results outlined above: the scrapie agent is not a virus but a proteinaceous infectious agent, which he called a "prion". The corresponding publication in *Science* was the birth of prions and, at the same time, the beginning of a worldwide controversy [5]. An infectious agent without a nucleic acid as information carrier was — and for some researchers still is — too much of a violation of a central dogma of molecular biology. It was argued that most experiments have lent only indirect support for the new agent, and one still might assume an unidentified virus with unconventional properties, which had prevented its identification.

If the agent consisted predominantly or solely of protein, the next task was to isolate and characterize the protein. It took several years and resulted in a single protein of 33–35 kDa, called the prion protein (PrP). It is glycosylated, has a C-terminal glycolipid anchor, is highly hydrophobic, and appears as a membrane-associated protein. After purifying the protein, Prusiner together with the laboratories of L. Hood and Ch. Weissmann identified and cloned the gene of the protein. Surprisingly or not, the gene of PrP is a single copy gene of the host [6]. A chemically identical protein was isolated either from healthy animals or from infected ones. Thus, one had to assume functional differences, so-called isoforms of PrP. In the healthy organism the cellular isoform PrP^{C} is produced, in the infected organism the abnormal or scrapie-form PrP^{Sc}, or PrP^{CJD} and PrP^{BSE} in the cases of CJD- and BSE-infections, respectively. The situation is depicted in the sketch in Fig. 1 of a healthy and an infected hamster. As a consequence of the infection, PrP^{C} is transformed into PrP^{Sc} which is then present in a much higher concentration. It is noteworthy that the golden hamster became the experimental animal of choice, since the incubation time after intracerebral injection is fairly short (around three months) and, even more important, it is strictly related to the titre of the infectious material.

In spite of an intensive search, no chemical differences such as differences in size, sequence or modification, could be found between PrP isolated from healthy animals or from infected animals [7].

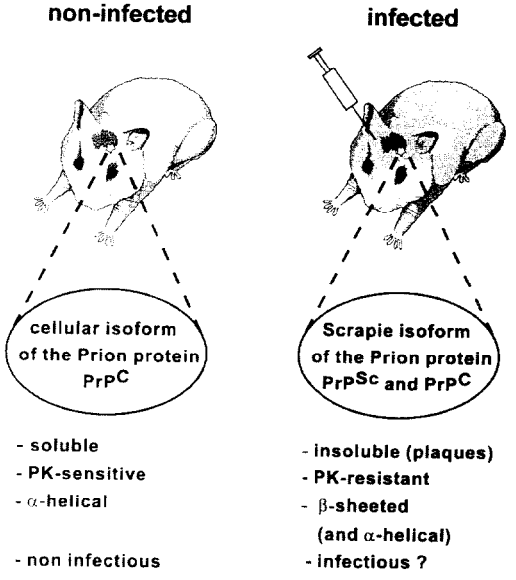

Fig. 1. Schematic presentation of prion protein isoforms.

3. Experiments pro and contra prions

What are the most obvious recent experiments to favour the prion model? Do other results exist which argue against the model, and why do some researchers still try to deny the existence of prions?

The $PrP^{o/o}$-mouse is a transgenic mouse in which both alleles of the PrP gene have been knocked out [8]. If such a mouse is inoculated with prions, no symptoms of a disease can be observed even after much longer incubation times than normal in mice, and the disease cannot be passaged further from this animal. Such a result had to be expected if PrP is the carrier of the pathogenesis as well as responsible for the infection. Thus, the finding is in complete agreement with the prion model but does not prove it, since it shows only that PrP is necessary but not that it is sufficient.

The phenomenon of scrapie strains is not so easily reconciled with the prion model. The principle of the experiments carried out by the Scottish scrapie researchers Dickinson and Kimberlin [9] is depicted in Fig. 2. Two different "isolates" of scrapie-infectious material — not considering here the origin of different isolates — were used to inoculate genetically identical hamsters. In the first passage a species barrier had to be overcome, and therefore the incubation time might be different after inoculation with different isolates. In the second passage, however, prions with an identical PrP-sequence were used for inoculation, and still clearly different incubation times and different lesion patterns in the brain were obtained. Prions seem to contain more information than the mere sequence of PrP. This phenomenon is called "prion strains", and several researchers take it as an indication of the presence of an informational molecule, most probably a hitherto undetected nucleic acid. The strain problem became of present day relevance when a new variant of CJD was identified in 15 patients in the U.K. in 1995 and 1996; the onset and progression of the disease were clearly different from those known so far with CJD. It is suspected that this new variant might be due to transmission of the BSE agent to humans via the food chain [10].

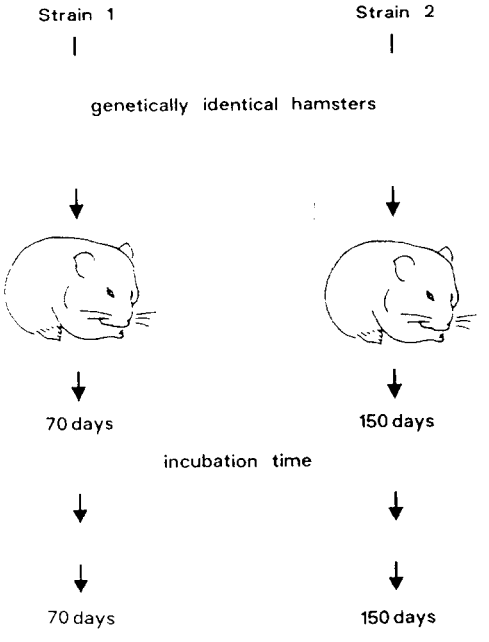

Fig. 2. Schematic presentation of scrapie strains. The arrows indicate passages.

At the end of the 1980s the existence of prion strains prompted us, in cooperation with Prusiner's laboratory, to undertake a direct and quantitative nucleic acid analysis which had not been carried out before in a rigorous way. The concept was to start with highly purified prions and perform two types of measurement: (i) size and numbers of nucleic acid molecules present in the infectious material, and (ii) number of infectious units. The ratio of the numbers of nucleic acid molecules and infectious units (P/I) might lead to two different conclusions. If the ratio were to be larger than unity, no strict conclusion would be possible. If, however, the ratio were to be smaller than unity, more infectious units would be present than nucleic acid molecules and therefore the nucleic acids would be excluded from being essential for infectivity. The numbers of infectious units were determined in a biotest using the incubation time assay, for the nucleic acid analysis a novel method, the so-called return refocussing gel electrophoresis, had to be developed. As outlined in the original literature, this technique was adapted to the very peculiar situation, i.e. that nothing was known about the nucleic acid to be analyzed, and DNA or RNA, double- or single-stranded etc. and even heterogeneous nucleic acid had to be taken into consideration [11]. As a summary of several experiments the P/I

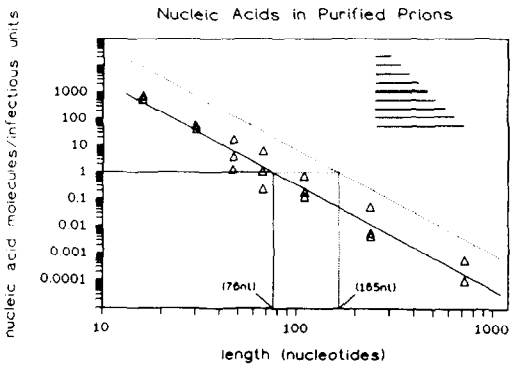

Fig. 3. Particle-to-infectivity ratio (P/I); dependence upon the length of the hypothetical scrapie-specific nucleic acid. A distinct molecular species (heavy line in the insertion) among the heterogeneous background nucleic acids (thin lines in the insertion) was assumed. Experimental values from several independent prion preparations were evaluated. The straight line is an interpolation of the experimental data. The dotted lines represent the maximum error. For details cf. [11]. Figure is modified from [11].

ratio in dependence upon the length of the nucleic found in the infectious material is given in Fig. 3. Only nucleic acids smaller than eighty nucleotides (fifty in recent as yet unpublished studies) are present in amounts with P/I >1, i.e. could still be essential for scrapie infectivity; larger nucleic acids, i.e. also those of viruses or viroids, are excluded. Thus, the nucleic acid analysis represents a completely independent proof that the agent of scrapie cannot be a virus [11].

4. Properties of the prion protein

Although differences in the chemical composition between PrP^{C} and PrP^{Sc} could not be found, differences in the secondary structure and related physico-chemical properties have been described. PrP^{C} is soluble under particular conditions, whereas PrP^{Sc} withstands all attempts at solubilization while keeping infectivity. Instead it forms large aggregates, which can be detected as plaques in the brain or as regular amyloidic structures after purification. An electron micrograph of such amyloidic aggregates is shown in Fig. 4a. The strong tendency to aggregate as well as the presumed simplicity of the agent might be the reason for its extraordinary heat stability. Autoclaving at temperatures of 134°C or even higher are required for complete deactivation [12]. Also a consequence of aggregation is most probably the resistance of PrP^{Sc} to proteinase K digestion after an initial truncation around amino acid 90, whereas PrP^{C} is degraded to oligopeptides. In SDS–gel electrophoresis three bands remain corresponding to the peptide 90–231 without glycosylation, with one, and with both glycosyl groups attached, respectively [13,15,16]. It should be noted that the material remains completely infectious after N-terminal truncation, and transgenic mice expressing only the peptide 90–231 can still be infected [14]. The band pattern may vary with different forms of the disease, the exact site of truncation as well as the relative intensities of the three bands. These properties

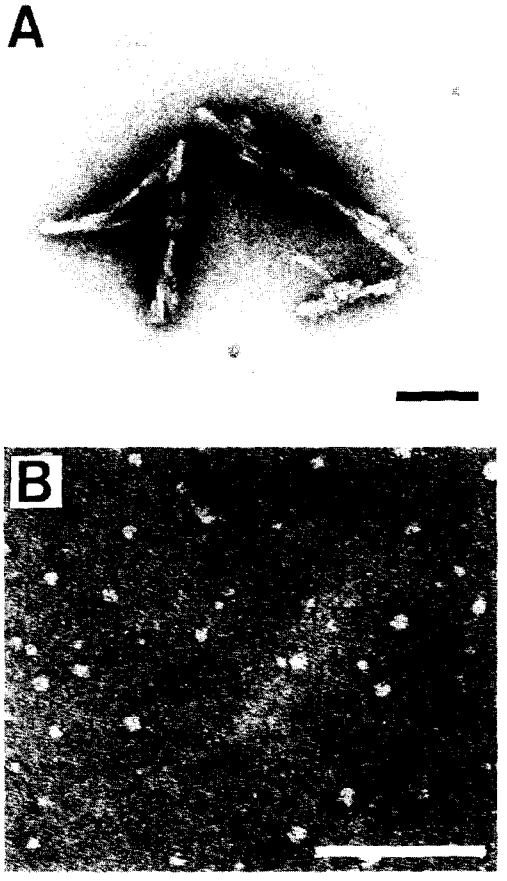

Fig. 4. Electron micrographs of prion rods (A) and spherical PrP27–30 particles after solubilization. The bars represent 100 nm. Figure is modified from [19].

were utilized for strain typing. Prusiner and his colleagues could show that these together with other strain properties breed through, even if the disease is transmitted from humans to transgenic mice [15]. Collinge and coworkers found the same pattern in BSE-infected cattle and patients of the new variant CJD supporting the suspicion of a BSE transmission to humans [16].

The secondary structures of PrP^C and to some extent of PrP^{Sc} are known from spectroscopic studies. IR and CD-spectroscopy yielded over 40% α-helicity without detectable β-sheets in PrP^C, but less than 30% α-helix and about 30% β-sheets in PrP^{Sc} [17–19]. The groups of Glockshuber and Wüthrich have determined the three-dimensional structure of a C-terminal fragment (AA 121–231) which was an *E. coli* expressed fragment [20]. As shown in Fig. 5, the structure consists of three α-helices and a small antiparallel β-sheet. It must be assumed that at least one of these helices is transformed into a β-sheeted motif during the $PrP^C \rightarrow PrP^{Sc}$ transition. However, only rough models are presently available depicting the PrP^{Sc} structure [21], and because of the insolubility the chances for structure determination by NMR or X-ray analysis in the near future are rather low.

Fig. 5. Structure of the recombinant C-terminal fragment of PrP from aminoacid 121 to 231 as analyzed by NMR. The figure is reproduced with permission from [20].

Having discussed the characteristic properties of PrP^C and PrP^{Sc}, one should emphasize two questions of nomenclature. The original prion hypothesis claimed that a protein is the predominant component of the agent. A simplified formulation is the so-called "protein-only hypothesis" which states that solely the prion protein in the scrapie isoform represents the infectious agent. Another matter of clear definition is the difference between PrP^{Sc} and PrPres, the first denoting PrP in scrapie infectious material, the second PrP in the conformation of being resistant to PK digestion. It should be emphasized that PrP^{Sc} denotes a biological and PrPres a biochemical property, respectively; they are correlated in many experiments but cannot be used as identities, which has unfortunately happened several times in the literature. The potential difficulties hidden behind the two definitions might become evident from the finding that about 10^5 PrP molecules are needed for one infectious unit. Are they all identical in conformation, and the incidence of infection so low? Or do much fewer PrP molecules exist which are the really infectious molecules PrP^{Sc} (sometimes also called PrP*), which are protected by 10^5 molecules PrPres. This is definitely an analytical question of protein chemistry, but at present no method is available to analyze a protein heterogeneity less than a percent. Protein heterogeneity in the form of a second amino acid sequence originating from a second mRNA would be detectable in very low abundance, but it was shown that no second mRNA of PrP with an abundance of more than 0.1% of the known sequence exists in the infected organism [22].

The structures of PrP^C and PrP^{Sc} are presently the basis for any models of prion replication. Only a very rough picture will be described here, and experimental details and more sophisticated models will be added later. In the non-infected organism PrP^C is expressed in concentrations varying from tissue to tissue, and is located on the outer membrane of the cell and anchored by its glycolipid anchor. It is degraded with a relatively high turnover rate. In the course of an infection, one might assume that the invading PrP^{Sc} comes into close contact with PrP^C, and due to this contact a conformational transition of PrP^C into PrP^{Sc} is induced. Induced conformational changes of proteins are known from chaperone action, and in that sense PrP might act as its own

chaperone. The transition of PrP^C to PrP^{Sc} which is induced by PrP^{Sc} represents an autocatalytic process, which might go on as long as new PrP^C molecules are synthesized in the cell. The newly generated PrP^{Sc} will form new aggregates, or be incorporated into already existing aggregates; it will be stabilized by aggregation, thereby acquiring a much longer turnover time. The size of aggregates is, however, not known.

5. Infectious, sporadic, familial

If the $PrP^C \rightarrow PrP^{Sc}$ transition is assumed to be the primary event of the disease, essential conclusions can be drawn or, if looked at from the other side, remarkable experimental findings might be explained on the basis of this assumption. Firstly, a conformational transition which is catalyzed by the contact with other proteins or factors should also occur spontaneously according to the laws of thermodynamics, although the probability and the rate might be very low. If only a single or a few PrP^C molecules undergo the transition, they could start the autocatalytic cycle, and from the cell where it happened an infectious process could spread through the whole body or organ. This could be an explanation for the sporadic manifestation of the disease which is well known for CJD. Close to 90% of all CJD patients are afflicted by the sporadic form; the incidence is about one case per million a year, a number which was found to be similar in all parts of the world. Secondly, if a protein has two or more structural alternatives, both of which are stable, or one stable and the other metastable etc., one should expect germ-line mutations which favour PrP^{Sc} over PrP^C. Favouring has not to be exclusive for PrP^{Sc} but could also be a higher incidence for the spontaneous $PrP^C \rightarrow PrP^{Sc}$ transition. Indeed, many prion diseases with hereditary manifestations are known, and all are connected with mutations in the PrP gene. They are called familial CJD, Gestmann–Sträussler–Scheinker disease, or familial fatal insomnia. The reader might refer to specialized review articles for all the features of homo- and heterocygosity, predisposition and prevalence and the broad spectrum of symptoms and course of the disease [23].

Finally one should mention that the sporadic form of the disease could also be initiated by a somatic mutation, which then would start an autocatalytic cycle for infecting the whole organism as described above. The sporadic as well as the familial forms of the disease had been transmitted to experimental animals — or in the case of medical accidents to humans — which finding supports the general concept of prion diseases. In summary, prion diseases are exceptional also in the sense that they can be manifested as infectious as well as sporadic or familial etiology. Thus, it is a great merit of the prion concept as well as convincing support for it, that diseases of quite different etiology can be reduced to a unique phenomenon.

6. Structural transitions of the prion protein

As mentioned above, prion replication is intimately related to the process that PrP^{Sc} invading the organism induces a post-translational transition of PrP^C into PrP^{Sc}. Thus we will give a brief outline of experimental results of conformational transitions of PrP and discuss in the next paragraph Manfred Eigen's recent calculations on the kinetics of the different mechanistic models of prion replication [24].

Beside studies on fragments of PrP and on recombinant PrP, experiments have been carried out on three natural forms of PrP: PrP^C, PrP^{Sc} and the N-terminal-truncated but still infectious form of PrP^{Sc} called PrP27–30. Physico-chemical investigations suffered from the fact that PrP^{Sc} and PrP27–30 are insoluble in an aqueous buffer so that spectroscopy cannot be applied without particular arrangements. One way to circumvent those restrictions is spectroscopy on thin films by circular dichroism [25] or on single aggregates by micro-beam infrared spectroscopy [26]; the other approach was to try solubilization of PrP^{Sc} or PrP27–30 [19].

Although different approaches led to comparable results, we will outline here some results from the solubilization approach. Among the many detergents applied for solubilization Guanidiniumhydrochloride (GnHCl) could be used if infectivity and PK-resistance was tested [27], but sarkosyl and SDS were found favourable for biophysical studies [19]. Sonication of purified prion rods which consist of

PrP27–30, in 0.2% SDS could break down the aggregates, partly into smaller but still insoluble aggregates and partly into soluble particles consisting of four to six PrP27–30 molecules. The electron micrograph in Fig. 4B shows the soluble fraction as fairly homogeneous spherical particles of 10–12 nm diameter. Further analysis in sucrose gradient centrifugation identified the soluble fraction as oligomeric PrP27–30 of a Mr of 100,000–120,000. Due to the solubilization, PrP27–30 lost its PK-resistance. In addition to the soluble and the insoluble, i.e. sedimenting fraction, a third fraction of varying yield was found in the meniscus of the gradient, which consisted of PrP aggregated with cellular lipids. Bioassays showed that the PrP aggregates in the sediment and the PrP–lipid aggregates in the meniscus fraction had retained infectivity, that, however, the soluble fraction did not exhibit infectivity which was significantly higher than background. Thus, soluble prions could not be prepared so far, which is in accordance with results from several other solubilization attempts.

The fractions of the solubilization experiments as well as other preparations of PrP have been analyzed for its secondary structure by circular dichroism and infrared spectroscopy. In fair agreement between different studies it was found that PrP^C is high in α-helix content, and very low in β-sheets (cf. also the structure from NMR analysis in Fig. 5) whereas in PrP^{Sc} and PrP27–30 the amount of α-helices is markedly lower and over 30% β-sheets were found. The secondary structure of solubilized PrP27–30 from prion rods (cf. above) appears very similar to that of PrP^C. Quantitative data are given in Table 1.

Table 1

Secondary structure of PrP in different isoforms

	α-helix (%)	β-sheet (%)
PrP^C	43	—
PrP^{Sc}	20	34
PrP27–30	29	31
$(PrP27–30)_{4–6}$	61	5

The structure of PrP can be affected by solvent conditions. As known for other proteins, GnHCl and urea denature the secondary structure of PrP, hexafluoro-isopropanol and SDS favour α-helical structure and acetonitrile favours β-sheeted structure. In several studies the prion-typical structure of PrP^{Sc} or PrP27–30 could be transformed into PrP^C-characteristic structure [17,19,28]. In accordance with the prion model the PK-resistance and the infectivity was lost during the transition, but is the transition in the other direction also possible in the test tube, i.e. can one transform PrP^C into PrP^{Sc}, which would be the ultimate proof of the prion model? Why could that not be achieved up to now? Several approaches have been reported in the literature.

Caughey and his colleagues incubated radiolabelled PrP^C with a large excess of PrP^{Sc} under partially denaturing conditions [27]. They could show that PrP^C acquired PK-resistance after renaturation, and could induce even strain-specific N-terminal truncation sites. However, because of the large excess of PrP^{Sc} it could not be tested whether infectivity was newly generated, thus possibly PrPres but not PrP^{Sc} was induced. Kaneko and colleagues induced a transition from soluble PrP^C into an insoluble, PK-resistant structure by a large excess of the PrP-peptide 90–145 [29]. PrP27–30 which was solubilized by SDS could be re-transformed into an insoluble state by adding 25% acetonitrile [19]. The secondary structure, too, was changed back to the characteristic β-sheeted structure of prions and partial PK-resistance was induced. However, acquired infectivity as a consequence of the conformational change was not found.

As a summary of the studies reported above, one has to conclude that the properties of β-sheeted structure, insolubility, and PK-resistance are found in prions but are not strictly correlated with infectivity. Whereas the PrP–lipid aggregates were infectious but partially PK-sensitive [19], PK-resistance could be induced by several methods without, however, acquiring infectivity. PK-resistance was correlated with aggregation and aggregation with β-structure, but these features are not sufficient for infectivity. At present it cannot be decided whether the right conditions for induced infectivity have not been found, a second, not yet identified component is still missing, or a principle feature of the $PrP^C \rightarrow PrP^{Sc}$ transition is not yet understood.

7. Models of prion replication

The models of prion replication which have been reported in the literature by Prusiner [13] and Lansbury [30] are models of PrP conformational transitions. They are hypothetical in the sense that firstly they are derived from the "protein-only hypothesis" and secondly assume conformational transitions occurring in PrP free in solution. Although these assumptions are probably oversimplifications, more realistic assumptions are not accessible for a quantitative discussion. Since Eigen undertook a quantitative comparison of different models, it is appropriate to discuss the models together with the kinetic evaluation of Eigen [24].

The model in Fig. 6A was proposed by Prusiner and colleagues and is also called the heterodimer model, since the critical step is the formation of a heterodimer of PrP^C and PrP^{Sc}, which transforms PrP^C to PrP^{Sc} similar to the enzymatic mechanism of an induced fit. More recently the model was specified in that respect, that a protein X has to be involved in the complex [31]. The reaction scheme represents a linear autocatalysis with an exponential formation rate of PrP^{Sc} (=B). Regarding an equilibrium PrP^C (=A) $\rightleftharpoons$ PrP^{Sc} (=B), B would be the favoured state, otherwise there would be no driving force for the catalytic turnover which at equilibrium is reversible. Eigen's calculations show that the spontaneous transformation of A into B has to be extremely low ($<< 10^{-22}\ s^{-1}$), otherwise B will grow spontaneously even without infection. However, to make an infection to work in a life span, one would have to assume an extreme enhancement of the $PrP^C \rightarrow PrP^{Sc}$ transition of more than 10^{15} due to heterodimer formation, which is not known from other enzymatic processes.

Eigen proposed an extension of the mechanism in Fig. 6A, the so-called "cooperative Prusiner mechanism", in which several molecules B have to cooperate to transform one molecule A and thus the catalytic effect of every single molecule B is smaller than in the heterodimer model. The scheme of this mechanism is presented in Fig. 6B according to an allosteric enzymatic mechanism with three ligand binding sites. Similarly to the mechanism of Fig. 6A, thermodynamic equilibrium favours state B over A, and the non-catalytic production of B is slow enough compared to metabolic removal, so that the concentration of B

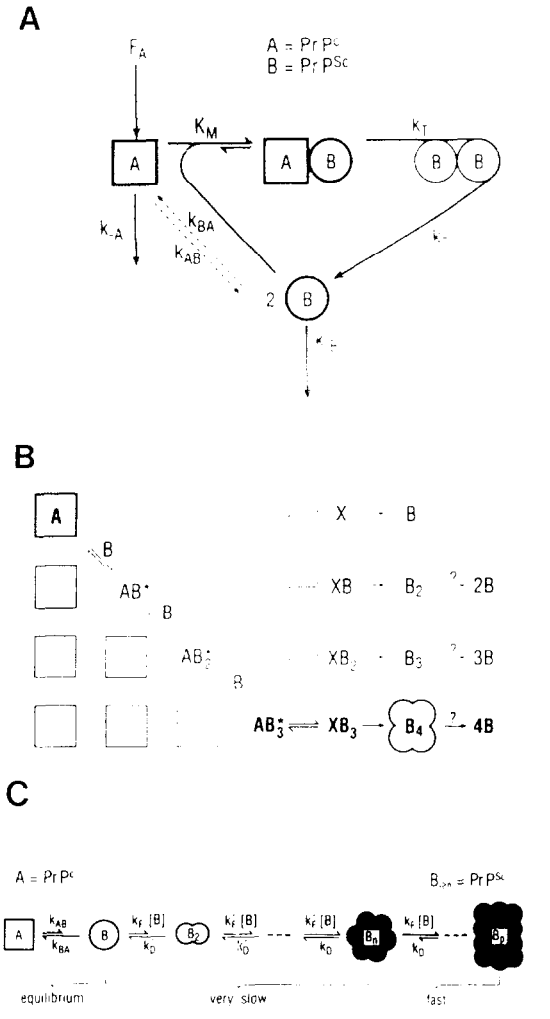

Fig. 6. Models of prion replication in the presentation of Eigen [24]: (A) Prusiner model; (B) cooperative Prusiner model; (C) Lansbury model. Figure is modified from [24].

cannot size to any appreciable level in the absence of infection. In contrast to the linear catalysis, however, the higher order catalysis leads to a threshold concentration for B corresponding to an infection event, which switches the system to the steady state of catalytic amplification of B.

Another mechanism was proposed by Lansbury [30], in which fibril formation as known for actin, β-amyloid etc., or called "linear crystals", and generation of infectivity are closely connected. As shown in the scheme of Fig. 6C, the species A and B are in a fast equilibrium, with A being the favourable state. B can

form aggregates B_i with decreasing concentrations down to a nucleus B_n; if the nucleus has been formed, growth of the aggregates is faster than dissociation, and indefinite aggregates will be formed. In contrast to the mechanisms of Fig. 6A and 6B, the molecule in state B does not represent a pathogenic form, only the nucleus B_n would represent an infectious entity. It was estimated that a very limited range of rate constants as defined in the scheme of Fig. 6C might exist so that the Lansbury mechanism could work.

In Eigen's article the pros and cons of the mechanisms are discussed in respect to the different types of catalysis, cooperativity, equilibria $A \rightleftharpoons B$, dissociation of B from the aggregates, etc. The reader should refer to the original article. At present we would like to point to a few biophysical questions which are evident from the experimental results as well as from the models.

Since the transition of PrP^C to PrP^{Sc} cannot yet be performed in the test tube, biophysical results on the structure and structural transitions of PrP will be rated as high, as they might lead to the desired *in vitro* $PrP^C \rightarrow PrP^{Sc}$ transition which then would represent the ultimate proof of the prion hypothesis. It will not matter whether a physical, a chemical, an enzymatic or any other treatment of PrP^C is applied as long as infectivity is newly generated from a non-infectious PrP^C sample. At present it is not clear (and the models start from different assumptions in that respect) whether the conformation of PrP^{Sc} exists already in the monomeric PrP, in an oligomeric state or how big the smallest size of the infectious entity might be. Infectious prions are only available as large aggregates; is this an artifact from the preparation or an intrinsic feature of the infectious state? How could the large aggregates be dissociated to generate more infectious particles, which is an essential question particularly with the Lansbury model? As mentioned above, most experiments and models refer to conditions in solution; it might be that the transition will work only in close contact with the membrane, i.e. closer simulation of cellular conditions would be required for a successful $PrP^C \rightarrow PrP^{Sc}$ transition.

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