REVIEW ARTICLE

Prion liposomes

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INTRODUCTION

Perhaps the novelty and insolubility of the scrapie agent have been the two factors which contributed most to the confusing and tortuous history of this particle. Scrapie is a degenerative neurological disease of sheep and goats [1–4]. Although scrapie can be transmitted to animals by inoculation with extracts of diseased tissue, whether infection features in the spread of natural scrapie is unclear [3–10]. Some investigators proposed that natural scrapie is a genetic disease and that its transmissibility is incidental [3,8] while others have argued vehemently that natural scrapie is an infectious disease [9,10].

Seven diseases, four in animals and three in humans, are thought to be caused by prions. The animal prion diseases are scrapie, transmissible mink encephalopathy [11], chronic wasting disease of mule deer and elk [12] and bovine spongiform encephalopathy [13,14]. The human prion diseases are kuru [15], Creutzfeldt–Jakob disease (CJD) [16] and Gerstmann–Sträussler syndrome (GSS) [17], which illustrate the transmissible, sporadic and familial manifestations of these disorders [18–20]. Consistent with some of the observations on natural scrapie noted above, GSS and familial CJD are the only known human diseases which appear to be both inherited and transmissible [18,21–24]. Recent genetic linkage studies provide the best evidence, to date, that GSS is a genetic disorder in which infectious prions are produced [18]. An alternative hypothesis, for which there are few supporting data, is that prions are ubiquitous infectious pathogens causing CJD sporadically across the earth at a rate of one case per million population and GSS in all individuals carrying a mutation in one allele of their PrP gene [18–21,25,26]. Our genetic linkage results with GSS raise the possibility that sporadic CJD arises from a somatic mutation [5,18,25].

While many exciting and provocative areas of prion research have evolved over the past 5 years, it is neither the intent nor province of this review to chronicle all these developments. Many other reviews [5,19,27–32] and books [33,34] provide more broad coverage of the subject. Instead, this review is intended to focus primarily on the recent advances in prion research that have come from the application of liposome technology.

ON THE DEFINITION OF PRIONS

Although the unusual properties of the particle causing scrapie were first appreciated in the 1940s [35], it was not

until a mouse bioassay was developed by Chandler [36] that convincing data were obtained to suggest that the scrapie agent might not be a virus, bacterium, fungus or parasite [37–39]. Over the decade from 1965 to 1974, radiobiological studies offered the most provocative data suggesting that the scrapie agent is fundamentally different from small pathogens such as viruses [37,38]. The unusual features of the scrapie agent led to suggestions that the scrapie agent might be a replicating membrane fragment [40,41], a viroid [42], a carbohydrate moiety [43,44], a protein [45–48] or even a small virus [49–52]. Terms such as ‘unconventional virus’ and ‘virino’ were introduced to distinguish the scrapie agent from viruses [1,53]. Although the term unconventional virus has been used widely, some investigators believe it is counterproductive to adopt such terminology. In surveying the status of research for the decade 1969–1978, Ian Pattison [54] recently wrote “...with the causal agent of scrapie still obscure, and virologists as adamant as ever that theirs was the only worthwhile point of view. To explain findings that did not fit with a virus hypothesis, they had rechristened the causal agent an ‘unconventional virus’. Use of this ingenious cover-up for uncertainty made ‘virus’ meaningless – for is not a cottage an unconventional castle?” Recently, unconventional viruses have been suggested to be mineral–protein complexes which multiply by crystallization and have been said to be similar to computer viruses [55,56].

‘Virino’ was defined: “If the recent experimental results of Marsh and Malone are correct in implicating DNA as a necessary component of the infective unit of scrapie, then an appropriate name for this class of agent would be ‘virinos’ which (by analogy with neutrinos) are small, immunologically neutral particles with high penetration properties but needing special criteria to detect their presence” [53]. Subsequent studies showed that the work of Marsh *et al.* [57] and Malone *et al.* [58] could not be confirmed [59]. The most recent definition of ‘virino’ is ‘host proteins sequestering the agent genome which may code for no product other than copies of itself’ [60].

While several studies raised the possibility that protein might be required for scrapie infectivity [61–63], convincing data showing that a protein molecule is necessary for infectivity were obtained only after an effective protocol for partial purification of the scrapie agent was developed [64]. Attempts to use the same protocol to demonstrate that the scrapie agent contains a nucleic acid were unsuccessful. Five procedures that modify or

Abbreviations used: CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler syndrome; PrP, prion protein; PrP^{Sc}, scrapie isoform of the prion protein; PrP^C, cellular isoform of the prion protein; mAb, monoclonal antibody; DLPC, detergent–lipid–protein complex; Tg, transgenic; GPI, glycosylphosphatidylinositol; PIPLC, phosphatidylinositol-specific phospholipase C; Sarkosyl, sodium dodecyl sarcosinate; SAF, scrapie-associated fibrils; PC, phosphatidylcholine; ORF, open reading frame.

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hydrolyse nucleic acids failed to inactivate the scrapie agent, yet these procedures were capable of inactivating numerous viruses as well as viroids [65,66]. On the basis of these studies, the term 'prion' was introduced in order to distinguish the class of particles causing scrapie from those responsible for viral illnesses [65].

To avoid prejudging the structure of these infectious particles, prions were defined as 'small proteinaceous infectious particles that resist inactivation by procedures which modify nucleic acids' [65] and three hypothetical structures for the prion were proposed: (1) proteins surround a nucleic acid that encodes them (a virus), (2) proteins surrounding a small non-coding polynucleotide, and (3) a proteinaceous particle devoid of nucleic acid. Data from many laboratories have established that scrapie is not caused by a virus. Although some investigators have chosen to redefine prions as infectious proteins [67,68], we have avoided this oversimplification in order not to bias our experimental approaches [69].

As described below, considerable data argue persuasively that a protein designated PrP^{Sc} is a major and necessary component of the infectious scrapie particle. Yet unlike viral proteins, PrP^{Sc} is encoded by a chromosomal gene. Based on these observations, the definition of prions [70] can be extended and stated as 'small proteinaceous infectious particles that resist inactivation by procedures modifying nucleic acids and contain an abnormal isoform of a cellular protein which is a major and necessary component.'

EVIDENCE THAT PRIONS CONTAIN PrP^{Sc}

Enriching brain fractions for scrapie infectivity led to the discovery of PrP 27-30 [71,72]. Development of a more rapid and economical bioassay [73,74] greatly facilitated purification of the hamster scrapie agent [71,72]. PrP 27-30 migrates during SDS/PAGE as a broad band with an apparent M_r of 27000-30000.

There has been a remarkable convergence of experimental data which argue that PrP^{Sc} (or PrP 27-30) is a component of the infectious prion particle (Table 1). Biochemical purification and characterization studies, molecular cloning and genetic investigations, neuropathological studies in animals and humans as well as immunoaffinity purification and neutralization all provide data supporting the contention that prions are composed largely, if not entirely, of PrP^{Sc} molecules. PrP 27-30 and the scrapie agent copurify [72,75-77] when using detergent extractions and limited proteolysis to promote aggregation of prions into amyloid rods which are collected by centrifugation. PrP 27-30 is the most abundant macromolecule in purified preparations [77]. Immunoaffinity purification of scrapie prions was accomplished with PrP monoclonal antibody (mAb) coupled to Protein A-Sepharose. Dispersion of prions into detergent-lipid-protein complexes (DLPCs) was required before meaningful chromatography could be performed [78,79]. Copurification of scrapie prion infectivity and PrP^{Sc} was found in fractions eluted from a PrP mAb column [80]. Indeed, copurification by two different procedures argues that the molecular structure and properties of PrP^{Sc} (as well as PrP 27-30) and the infectious prion particles must be extremely similar. The PrP 27-30 concentration is proportional to the prion titre [75]. PrP^{Sc} is absent from normal uninfected animals [94,95]. Procedures that denature, hydrolyse or

Table 1. Convergence of experimental results arguing that PrP^{Sc} (or PrP 27-30) is a major and necessary component of the prion

A. Biochemistry

1. Enriching brain fractions for scrapie infectivity using limited proteolysis, detergent extraction, differential centrifugation and sedimentation through discontinuous sucrose gradients led to the discovery of PrP 27-30 [71,72].
2. Prion titres were found to be proportional to the PrP 27-30 concentration [75].
3. Denaturation, hydrolysis or selective modification of PrP 27-30 diminished prion titre [75,76].
4. Scrapie infectivity and PrP 27-30 copartition into multiple forms — membranes, rods, spheres, DLPCs and liposomes [72,77-79].

B. Immunology

1. Immunoaffinity purification of scrapie infectivity was accomplished using PrP mAbs [80].
2. Rabbit antiserum raised against SDS/PAGE-purified PrP 27-30 neutralized scrapie infectivity in DLPCs [80].

C. Genetics

1. Genetic linkage of the PrP gene to loci controlling the scrapie incubation time (*Prn-i* and *Sinc*) [81,82]. Mice (*Prn-p*) of long incubation times exhibit amino acid substitutions of codons 108 and 189 of their PrP gene [83].
2. Genetic linkage of an amino acid substitution at codon 102 of the human PrP gene with development of GSS [18,84].
3. Transgenic mice expressing hamster PrP exhibit incubation times, amyloid plaques, hamster PrP^{Sc} and scrapie infectivity characteristic of hamsters after inoculation with hamster prions [85].

D. Neuropathology

1. PrP amyloid plaques are specific to prion diseases in animals and humans [86-88].
2. Hamster prions inoculated into Tg mice expressing hamster PrP induce cerebral amyloid plaques that are composed of hamster PrP and distributed in patterns similar to those seen in hamsters with scrapie [85].
3. Using a sensitive dot-blot assay for PrP^{Sc} (or PrP^{CJD}), there is an excellent correlation between prion diseases confirmed neuropathologically and the presence of protease-resistant PrP [89].

E. Cell biology

1. Cultured cells infected with scrapie prions produce PrP^{Sc} [90].
2. PrP^{Sc} is produced more slowly than PrP^C; furthermore, PrP^{Sc} accumulates primarily inside of cells while most of the PrP^C is transported to the external cell surface [91-93].

selectively modify PrP 27-30 also diminish the prion titre [75,76]. The unusual kinetics of PrP 27-30 hydrolysis catalysed by proteases were found to correlate with the diminution of prion titre. PrP 27-30 and scrapie infectivity copartition into many different forms — membranes, rods, spheres, DLPCs, and liposomes. These dramatically different physical forms all contain PrP 27-30 and high prion titres [72,77,78,93,96-98]. Rabbit antisera raised against PrP 27-30 purified by SDS/PAGE were found to neutralize scrapie infectivity in DLPCs [80]. However, no neutralization of prion infectivity was observed with prions aggregated in amyloid rods [99,100].

The PrP gene (*Prn-p*) in mice is linked to a gene controlling scrapie incubation times (*Prn-i*) [81]. Pro-

longed incubation periods are a cardinal feature of both scrapie and CJD. The pre-eminent role of PrP in the pathogenesis of scrapie has been made more compelling by the discovery of a correlation between PrP amino acid sequence and scrapie incubation times [83]. An amino acid substitution in the human PrP gene is linked genetically to the development of GSS [18,84]. GSS and familial CJD are the only known human diseases which are both genetic and infectious.

Recently, transgenic (Tg) mice expressing Syrian golden hamster PrP^C have been produced [85]. The Tg mice inoculated with hamster prions synthesize both hamster PrP^{Sc} and infectious hamster prions; these results assert that PrP^{Sc} is a necessary component of the infectious prion. Furthermore, the Tg mice exhibit scrapie incubation times and neuropathology characteristic of hamsters. Numerous amyloid plaques containing hamster PrP were found in the brains of the Tg mice in a distribution similar to that found in hamsters.

Scrapie and CJD prion proteins have been identified only in tissues of animals and humans with transmissible neurodegenerative diseases and not in those with other disorders, such as murine systemic amyloidosis and human Alzheimer's disease, anoxic encephalopathy or non-neurological disorders [72,74,76,77,86,87,101–103].

Amyloid plaques in prion diseases were first identified in the brains of New Guinea natives dying of kuru [104]. Subsequently, they were reported in scrapie and chronic wasting disease of animals [105–109]. With antisera raised against PrP 27–30 isolated from scrapie-infected hamster brain, central nervous system amyloid plaques in experimental scrapie were shown to be composed of filaments containing PrP [88,110]. Similar studies with brain sections from humans dying of kuru, CJD and GSS were also found to contain amyloid plaques which react with PrP antibodies [86,87]. To date, amyloid plaques reacting with PrP antibodies have been found only in prion diseases.

Cultured murine neuroblastoma cells have been infected with both scrapie and CJD prions [90,111,112]. Clones of the scrapie-infected cells were found to produce PrP^{Sc}, whereas clones showing no infectivity lacked PrP^{Sc} [90]. In scrapie-infected cultured cells, PrP^C is synthesized rapidly ($t_{1/2} \leq 2$ h) [113] and degraded with a $t_{1/2}$ of ~ 5 h while PrP^{Sc} is synthesized slowly ($t_{1/2} \sim 15$ h) and does not appear to be degraded [93]. Most PrP^C is transported to the external cell surface, where it is bound by a glycosylphosphatidylinositol (GPI) anchor, but can be released by digestion with phosphatidylinositol-specific phospholipase C (PIPLC) [91]. In contrast, PrP^{Sc} accumulates largely inside of cells, probably within the Golgi apparatus, and is not released from membranes by PIPLC [92]. The contrasting properties of PrP^C and PrP^{Sc} are listed in Table 2.

Many investigators have confirmed the presence of protease-resistant PrP in brains infected with the scrapie or CJD agent [103,114–117]. Although the amino acid sequence of PrP has been confirmed and there is agreement that PrP is glycosylated, some investigators have suggested that PrP 27–30 may not be a component of the scrapie agent [118,119]. One argument revolves around the inability of some investigators to detect PrP mRNA or PrP^{Sc} in spleens of scrapie-infected rodents [120,121]; however, investigators in several laboratories have clearly shown that both PrP mRNA and PrP^{Sc} are present in spleen tissue [95,122–124]. Indeed, recent studies have

Table 2. Properties of cellular and scrapie PrP isoforms

	PrP ^C	PrP ^{Sc}
Normal cells	$\sim 1\text{--}5 \mu\text{g/g}$	–
Scrapie-infected cells	$\sim 1\text{--}5 \mu\text{g/g}$	$\sim 10\text{--}20 \mu\text{g/g}$
Purified prions	–	+
Protease resistance	–	+
Amyloid rods	–	+
Subcellular localization	Cell surface	Primarily intracellular
PIPLC release from membranes	+	–
Synthesis ($t_{1/2}$)	≤ 2 h	~ 15 h
Turnover ($t_{1/2}$)	~ 5 h	≥ 24 h

* Copurification of PrP^{Sc} and prion infectivity demonstrated by two protocols: (1) detergent extraction, sedimentation, protease digestion, and (2) PrP 27–30 monoclonal antibody affinity chromatography.

† Limited proteinase K digestion of hamster PrP^{Sc} produces PrP 27–30.

‡ After limited proteolysis of PrP^{Sc} to produce PrP 27–30 and detergent extraction, amyloid rods form; except for length, the rods are indistinguishable from amyloid filaments forming plaques.

shown an excellent correlation between the concentration of PrP mRNA in scrapie-infected rodent tissues and prion titre [122]. Another argument centres on experiments that demonstrate the loss of CJD infectivity in fractions following lectin chromatography. Neither denaturation of the PrP CJD nor neutralization of CJD infectivity by immobilization on the lectin column was considered as an explanation [117]. Denaturation of PrP^{Sc} has been demonstrated to be accompanied by a loss of scrapie infectivity [76,77]. Still another argument involves the inability of some investigators to detect PrP 27–30 in hamster brains just prior to clinical signs of scrapie even though high prion titres are found at this time [119]. Unfortunately, these investigators determined prion titres from homogenates but measured PrP 27–30 in partially purified fractions. Other investigators have measured both prion titres and PrP 27–30 in crude extracts and have found an excellent correlation throughout the course of infection (R. E. Race, personal communication). Indeed, no experimental studies have been reported where fractions with high levels of scrapie infectivity were found to contain less than one PrP^{Sc} (or PrP 27–30) molecule per infectious unit.

EARLY ATTEMPTS AT PURIFICATION OF SCRAPIE INFECTIVITY

Early attempts to purify the scrapie agent from sheep and goat tissues were frustrating and ineffective [2]. Equally frustrating were later studies using scrapie-infected mouse brains [2,63,125,126]. Although these studies demonstrated the association of the scrapie agent with membranes and the resistance of scrapie infectivity to many detergents, no effective solubilization procedure was devised [126,127]. This association of scrapie infectivity with membrane-rich fractions led to an elaborate scheme embodying the structure and replication of the scrapie agent which was called the 'membrane hypothesis' [40,41]. So ineffective were detergents in separating

scrapie infectivity from membranes that Hunter and coworkers concluded that 'In terms of the membrane hypothesis the agent can be defined as the smallest piece of affected membrane which has full biological activity in the mouse assay system' [128]. They also wrote: 'Indeed, if the membrane hypothesis proves to be generally valid, purification of the scrapie agent in the classical sense is impossible. Even if currently available methods of membrane fractionation are improved, the degree of concentration possible will be limited to one or two orders of magnitude by the sheer bulk of scrapie-affected membrane' [129].

Subsequently, many other investigators tried to purify scrapie infectivity or identify a subcellular fraction enriched for the agent. In general, the results of these studies were disappointing and they served only to reinforce the view that scrapie agent purification was 'impossible'. A variety of protocols were used in attempts to purify the scrapie agent. One involved copurification of the scrapie agent and microsomes [130]. Another involved isolation of a 'membrane-free' fraction after prolonged ultracentrifugation [131]. This fraction contained 1–10% of the scrapie agent and was precipitated with $(\text{NH}_4)_2\text{SO}_4$ before SDS/gel electrophoresis. After electrophoresis, prions were eluted from the gel in order to obtain a further purification [58]. Infectivity of a fraction eluted from an SDS electrophoretic gel was diminished nearly 10^3 ID₅₀ units by DNAase digestion [57,58]. These results were puzzling since the residual SDS in the gel eluates was probably sufficient to inactivate the DNAase [132]. By another procedure, DNAase digestion of a fraction containing less than 0.01% of the initial scrapie infectivity from a hydroxyapatite column diminished scrapie agent titre by $10^{1.5}$ ID₅₀ units. Although the results of these studies seemed encouraging initially, subsequent work failed to confirm the findings [59].

Cho and coworkers attempted to isolate the scrapie agent using a variety of detergent extractions after vigorous homogenization [133]. Their protocol produced fractions with numerous particles measuring 14 nm in diameter [134]. After much interest, these particles were subsequently shown to be ferritin [135].

Using equilibrium sucrose and NaCl density gradients, Siakotos and coworkers [136] attempted to purify the scrapie agent from murine brain. They suggested that there was a peak of infectivity at a sucrose density of 1.19 g/cm³. However, multiple peaks of infectivity were found throughout the gradients, indicating considerable heterogeneity of the agent with respect to density. These results showed that density-gradient centrifugation, when applied to crude suspensions of membranous material from brain, is probably not useful in isolating the scrapie agent. Other studies from the laboratory of Gajdusek and Gibbs demonstrated considerable heterogeneity of prions in metrizamide and CsCl density gradients [137]. None of the protocols described above led to the identification of a macromolecule within the scrapie prion.

PURIFICATION OF PRION AMYLOID RODS

Our own purification studies began with characterization of the sedimentation properties of scrapie infectivity assayed by end-point titrations in mice [138]. These studies established that the scrapie agent could exist as a

continuum of sizes and we postulated that this was due to hydrophobic interactions [139]. In contrast with Hunter and coworkers, we concluded that the monomeric scrapie agent must be quite small, in the range predicted by Alper and her colleagues using ionizing radiation to inactivate the infectivity [38]. But, like Hunter and colleagues [126,127], we were unable to identify any 'magical' detergent which completely dissociated the infectivity into a uniform species which could be readily purified [73]. Instead, we took advantage of: (1) the protease resistance of the prion, (2) its ability to withstand repeated detergent extraction and (3) its propensity to aggregate [59,64,72].

Our purification studies were greatly accelerated by the development of a reliable incubation time bioassay [74] using Syrian hamsters [140–142]. A protocol was developed using agarose-gel electrophoresis in the presence of sodium dodecyl sarcosinate (Sarkosyl) to isolate aggregated fractions from the top of the gel, which were enriched for scrapie prions about 10^2 -fold with respect to protein [59,64]. Those partially purified fractions could be inactivated by protease digestions, providing the first convincing data for protein within the infectious particle [64]. As noted above, earlier studies have suggested the existence of such a protein.

Subsequently, we replaced the electrophoretic step with a discontinuous sucrose gradient [72] which allowed: (1) enrichment of scrapie infectivity about 10^3 -fold, (2) identification of the scrapie prion protein (PrP 27–30) and (3) large-scale purification [77]. Rapidly sedimenting fractions contained: (1) high prion titres, (2) one protein, PrP 27–30 and (3) numerous rod-shaped polymers (see Fig. 1a) [72,77]. These rods were: (1) indistinguishable from purified amyloids ultrastructurally, (2) bound Congo Red and (3) exhibited green–gold birefringence [77]. Diringer and coworkers modified and miniaturized our purification scheme and confirmed the unusual properties of PrP 27–30 [115]. However, as discussed by T. O. Diener [32]: "Based on a series of unsupported assumptions, these investigators claimed that the prion rods were identical to scrapie-associated fibrils (SAF) and that SAF are composed of PrP 27–30. They did not address the issue that SAF had been repeatedly distinguished from amyloid based on their ultrastructure and tinctorial properties. Despite claims to the contrary, there is no convincing study which reports the composition of SAF; whether or not authentic SAF contain PrP 27–30 or PrP^{Sc} is unknown."

Recent studies have shown that prion rod formation requires both detergent extraction and limited proteolysis [143]. Membrane fractions isolated from scrapie-infected hamster brains in the presence of protease inhibitors failed to show any elongated structures upon detergent extraction. However, removal of the protease inhibitors followed by limited proteolysis led to prion rod formation upon detergent extraction. These results suggest that amyloid filaments in brains of animals and humans with prion diseases are composed of PrP^{Sc} (or PrP^{CJD}) molecules which are partially hydrolysed. One recent study equates the prion rods to SAF and claims that these structures are found *in vivo* within the brains of scrapie-infected mice [144]. These investigators examined detergent extracts of subcellular fractions, but significant proteolysis of PrP^{Sc} is evident on Western blots. Other studies report SAF in purified preparations of PrP^{Sc}; however, PrP^{Sc} was separated from other proteins prior

to *N*-terminal sequencing by size exclusion chromatography [116,145]. Presumably, a portion of the PrP is PrP 27–30 which was separated from PrP^{Sc} by size exclusion chromatography after SDS denaturation dissociated the rod-shaped structures observed by electron microscopy. The same explanation seems applicable to our own earlier studies where microsomal membranes prepared without protease inhibitors were thought to produce prion rods upon detergent extraction [98]. Our findings showing that limited proteolysis of PrP^{Sc} is required for polymerization into amyloid rods are of interest with respect to Alzheimer's disease cerebral amyloids in blood vessels and plaques of a small peptide derived by limited proteolysis from a much larger precursor protein [146,147].

INSOLUBILITY OF PRIONS

Although the insolubility of the prion rods prevented many studies on the structure of the infectious prion particle, it did facilitate purification of the rods. On the other hand, the availability of purified fractions containing the rods allowed: (1) characterization of many properties of the prion, (2) determination of the *N*-terminal sequence of PrP 27–30 from which numerous molecular biology and genetics studies emerged, (3) study of the covalent structure of PrP 27–30 and (4) production of antibodies.

Numerous procedures were examined for their effectiveness in disrupting the prion rods while preserving infectivity. These included detergents, salts, pH and

sonication, but none was successful [76,97]. On the other hand, denaturation with SDS and boiling, strong chaotropes, acid and base disrupted prion rod structure but simultaneously reduced scrapie infectivity [63,126,148].

Manuelidis and coworkers [149] have claimed solubilization of prions after detergent extraction of membranes followed by prolonged sonication and exposure to pH 8.9. Their criterion for solubilization was lack of sedimentation at 20000 *g* for 30 min. The supernatant fraction was reported to contain membrane fragments and CJD infectivity. We doubt that significant solubilization was achieved under these conditions.

Another protocol developed by Semancik, Marsh and their coworkers involved prolonged ultracentrifugation to isolate a membrane-free fraction [131]. They estimated the size of the membrane-free scrapie agent to be about 2 S. Unfortunately, the vast majority of scrapie infectivity was found in the pellet and it was unclear whether the supernatant infectivity represented contamination from the pellet.

SOLUBILIZATION OF PRION RODS WITH LIPIDS AND DETERGENTS

The discovery of conditions for solubilization of non-denatured PrP 27–30 was an important advance [78]. Purified prion rods were mixed with a combination of detergent and phospholipid to form DLPCs upon sonication. The DLPCs could be centrifuged at 170000 *g* for 30 min with most of the PrP 27–30 and scrapie

Fig. 1. Electron microscopy and dynamic light scattering of prion rods and liposomes

(a) Typical prion rods negatively stained with uranyl formate (bar = 100 nm). (b) Light-scattering profile of rods determined with laser particle analyser (model NY, Coulter Electronics). (c) Negatively stained liposomes containing PrP 27–30. (d) Light-scattering profile of liposomes.

infectivity remaining in the supernatant fraction. Removal of detergent by dialysis produced closed liposomes. Scrapie infectivity generally increased 10–100-fold after dissociation of the rods into DLPCs or liposomes. Electron micrographs of the prion rods and liposomes are shown in Figs. 1(a) and 1(c). Laser light-scattering studies showed that the DLPCs have a mean diameter of 20 nm while the rods have an effective diameter > 100 nm (Figs. 1b and 1d).

No difference in solubilization of prions was seen when Triton X-100, a non-ionic detergent, was used in place of the anionic detergents cholate and Sarkosyl. Purified lecithin, which contains mostly phosphatidylcholine (PC), was used in most studies, but other phospholipids, including phosphatidylethanolamine, phosphatidylserine and dolichol, were assessed for their ability to solubilize PrP 27–30 and preserve prion titre [79]. No significant differences among the detergents and phospholipids examined were found.

SOLUBILIZATION OF PRIONS FROM CELLULAR MEMBRANES

Solubilization of scrapie prion infectivity bound to cellular membranes was accomplished by applying the same technique which was used to solubilize PrP 27–30 in rods. Microsomal membranes from scrapie-infected hamster brain were then extracted with a combination of Sarkosyl and lecithin followed by DLPC formation upon sonication [79]. Dispersion of PrP^{Sc} into DLPCs preserved scrapie infectivity and allowed PrP^{Sc} to be fractionated and studied as a soluble molecule. The advantage of this protocol is that it avoids aggregation of PrP^{Sc} and permits solubilization of prion infectivity directly from the membranes.

IMMUNOAFFINITY PURIFICATION AND NEUTRALIZATION OF PRIONS

Having developed a protocol to disperse prion rods into DLPCs with full retention of infectivity and having adapted this protocol to permit DLPC formation directly from liposomes, we sought to utilize this solubilization procedure in purification of prion infectivity. We reasoned that PrP mAb-affinity chromatography would copurify solubilized PrP^{Sc} and prion infectivity if PrP^{Sc} were a component of the infectious scrapie particle. On the other hand, immunoaffinity chromatography should separate PrP^{Sc} from infectivity if the two were unrelated, as suggested by some investigators [117,118,121,144,149,150].

The microsomes were solubilized by a combination of Sarkosyl (2%, w/v) and PC (5 mg/ml) [80]. The DLPCs formed by this procedure were subjected to ultracentrifugation and the supernatant applied to PrP mAb affinity matrix. mAbs raised against PrP 27–30 [94] were cross-linked to Protein A–Sepharose in order to minimize the leakage of the antibodies. After an overnight incubation at 4 °C, the immunoaffinity matrix was washed with buffers containing increasing concentrations of salt and different detergent as well as 2% (w/v) PC. The PC was included in order to prevent aggregation of PrP^{Sc} while bound to the matrix and during elution (Fig. 2).

The elution of PrP from the matrix was accomplished with increasing concentrations of alkali. The pH of the

Fig. 2. Immunoaffinity copurification of PrP^{Sc} and prion infectivity

eluate was increased progressively from 9.5 to 11.2. At pH 9.5, the first detectable PrP was eluted. Acid was also examined but it was not efficient in eluting the prion protein. Because alkali is known to inactivate scrapie infectivity [148], samples were titrated immediately after elution to pH 7.

Selected fractions of the immunoaffinity purification procedure were analysed by SDS/PAGE and bioassay (Fig. 3). Most of the unbound PrP was eluted in the void volume; that not all of the PrP molecule are bound to the matrix may result from some PrP molecules possessing a configuration which is unfavourable for binding. No PrP was detected in subsequent washes prior to increasing the pH of the eluate buffer to 9.5. Approx. 10% of the PrP was recovered in the alkaline eluate; this represents ~20% of the PrP in the microsomal fraction. An equal amount of PrP was found in the flow-through fraction. At least 20% of the unaccounted-for PrP remained bound to the column and was eluted by additional washes with the pH 11.2 buffer. The extent of PrP purification was approx. 5700-fold and the purity of the PrP as judged by SDS/PAGE with silver staining was excellent. Similar results were obtained when the IgG fraction of a polyclonal rabbit anti-(PrP 27–30) serum was coupled to Protein A–Sepharose and used in place of the PrP mAb columns.

Aliquots of fractions from the PrP immunoaffinity column were inoculated into hamsters for bioassay of scrapie prion titre. Those fractions which contain PrP^{Sc} also contain scrapie infectivity, while those fractions with no detectable PrP^{Sc} contain either low levels of scrapie prions or none (Fig. 3a). Moreover, the amount of PrP^{Sc} recovered from the column was a function of the eluate pH and was roughly proportional to the prion titre. Although the specific infectivity (ID₅₀ units/mg of protein) increased by 4000-fold during purification, the infectivity per µg of PrP remained constant. We recovered ~4% of the total infectivity in the pH 11.2 eluate; this corresponded to ~10% of infectivity found in the microsome fraction. An additional ~10% of the microsome infectivity was found in the flow-through fraction.

Fig. 3. Immunoaffinity purification of prions

Microsomes isolated from 15 scrapie-infected hamster brains were solubilized by a combination of Sarkosyl and PC. The resulting DLPCs were incubated overnight at 4 °C with (a) PrP mAb (13A5) or (b) polyclonal rabbit anti-HIV GP120 synthetic peptide antiserum coupled to Protein A-Sepharose. The resin was then loaded into a column and washed sequentially with the following buffers: (1) 50 mM-Tris/HCl (pH 8.2)/0.5 M-NaCl/1% (w/v) Sarkosyl/2 mg/ml PC (four washes); (2) 50 mM-Tris/HCl/0.5 M-NaCl/0.5% (w/v) sodium deoxycholate/1 mg/ml PC (three washes). Each wash was equivalent to one column volume of 15 ml. The PrP^{Sc} bound to the resin was eluted by addition of alkali. Aliquots of selected fractions were analysed by bioassays for scrapie infectivity. Fractions listed on the horizontal axis are: H, homogenate; M, microsomes; DLPC, detergent-lipid-protein complex; F-T, flow-through; W-3, wash 3; W-10, wash 10; 9.5, pH 9.5 eluate; 10.0, pH 10.0 eluate; 11.2, pH 11.2 eluate.

What proportion of the unaccounted-for infectivity remained bound to column and how much was inactivated during alkali elution is unknown. The imprecision of the animal bioassay [74] for scrapie infectivity complicated attempts to determine accurately the degree of purification and the recovery for a particular step.

In order to establish the specificity of the immunoaffinity

purification protocol, we constructed an immunoaffinity matrix using an unrelated antibody raised against a synthetic peptide of GP120 of HIV. No PrP^{Sc} was eluted from the column regardless of the buffer pH, establishing that the binding of the PrP^{Sc} to the column was not nonspecific. Aliquots of the fractions from this experiment were bioassayed in hamsters. Prion infectivity was recovered only in the flow-through; none was found in fractions eluted with alkali (Fig. 3b). Comparing the recoveries of prion infectivity eluted by alkali for the two columns revealed an impressive difference of $\sim 10^8$.

While PrP mAbs were incapable of neutralizing scrapie infectivity, we found that a polyclonal rabbit antiserum raised against SDS/PAGE-purified PrP 27–30 could neutralize scrapie infectivity [80]. Preimmune serum failed to alter prion titre, whereas increasing concentrations of immune serum caused a progressive decrease in titre.

The immunoaffinity purification and neutralization results provided direct immunological and chromatographic demonstrations of a link between PrP^{Sc} and prion infectivity. These experiments could not have been done without functional solubilization of scrapie prions into DLPCs [78,79].

STRUCTURE AND ORGANIZATION OF PrP GENES

Oligonucleotides corresponding to a portion of the *N*-terminal amino acid sequence of PrP 27–30 [151] were synthesized and used to identify a PrP cDNA [95,120]. Southern blotting with PrP cDNA revealed a single-copy gene with the same restriction patterns as those in normal and scrapie-infected DNA from hamster brains. Unexpectedly, PrP mRNA was found at similar levels in both normal and scrapie-infected hamster brains [95,152].

Molecular cloning of the hamster PrP cDNA led to elucidation of the organization and structure of the hamster PrP gene; the entire open reading frame (ORF) or protein coding region is contained within a single exon [153]. The 5' end of the PrP gene contains multiple GC-rich initiation sites. An intron of ~ 10 kb separates exons I and II. Six lines of evidence argue that the two PrP isoforms have the same amino acid sequence. (1) No evidence for rearrangement of the PrP gene in scrapie has been found [95,153]. (2) The organization of the PrP gene provides no possibility for alternative splicing within the ORF [153]. (3) Only one PrP mRNA of 2.1 kb has been detected and its concentration does not change throughout the course of scrapie infection in hamster brain [95]. (4) 70% of PrP^{Sc} and 87% of PrP 27–30 have been sequenced by gas-phase protein sequencing (D. Teplow, D. Groth, L. Hood & S. B. Prusiner, unpublished work). These sequences correspond precisely to the translated genomic DNA sequence. (5) Both scrapie hamster brain and mouse PrP cDNAs have translated ORF sequences which are identical with the corresponding translated genomic sequences [83,95,153,154]. (6) Both PrP isoforms have the same *N*-terminal amino acid sequence as determined by *N*-terminal gas-phase sequencing [155]. Presumably, the difference in the properties of the two prion proteins is due to a post-translational event [93,153]; though seemingly remote, the possibility that differences in the amino acid sequences of the two PrP isoforms arising through RNA editing must continue to be considered [156–159].

That PrP genes existed prior to the speciation of

mammals is supported by the finding that human and mouse PrP genes are located on homologous chromosomes 20 and 2 [160]. The ORF of PrP genes from humans [161], Syrian hamsters [153], Chinese hamsters [162], Armenian hamsters [162], mice [83,154], rats [163] and sheep [164] have been sequenced and all encode prion proteins of approx. 250 amino acids with *N*-terminal signal peptides. All of these PrP ORFs encode *C*-terminal hydrophobic peptides which are presumably removed upon GPI anchor addition and all contain two consensus sites for Asn-linked glycosylation as well as two cysteines within the *C*-terminal half of the molecule. All of the ORFs also possess a series of glycine-rich repeats in the *N*-terminal portion of the PrP molecule. Whether eukaryotes other than mammals have authentic PrP genes remains to be established [165].

POST-TRANSLATIONAL MODIFICATIONS OF PRION PROTEINS

There is evidence for at least eight post-translational modifications of PrP (Fig. 4). The first modification to be identified was glycosylation; PrP 27–30 is a sialoglycoprotein [151,166]. All translated PrP gene ORFs sequenced to date contain two consensus sites for an Asn-linked glycosylation. In Syrian hamster PrP, these consensus sites are codons 181 and 197 [95,153]. Both the cellular and scrapie PrP isoforms are resistant to digestion by endoglycosidase H but possess *N*-linked oligosaccharides that can be removed by digestion with peptide: *N*-glycosidase F [167]. Hydrazinolysis liberated ~ 2 mol of

Fig. 4. Prion protein structure

Hamster PrP gene encodes a protein of 254 amino acids. The *N*-terminal signal peptide of 22 amino acids (stippled pattern) is cleaved during maturation of PrP^c and PrP^{sc}. Modified Arg residues (X) at codons 25 and 37 have been identified as well as Asn-linked oligosaccharides (CHO) attached at codons 181 and 197 within a loop formed by a disulphide bond which joins Cys at codons 179 and 214. Upon removal of a *C*-terminal hydrophobic peptide of ~ 20 amino acids (diagonal line pattern), a glycosylphosphatidylinositol (GPI) anchor is added. Reproduced, with permission, from Ann. Rev. Microbiol. 43, © 1989 Annual Reviews Inc.

oligosaccharides per mol of PrP 27–30 [168]. The released oligosaccharides were found to be a mixture of bi-, tri- and tetra-antennary complex-type sugar chains with Man α 1→6(GlcNAc β 1→4)(Man α 1→3)Man β 1→4-GlcNAc β →4 (Fuc α 1→6)GlcNAc as their cores. Variation is produced by combinations of the following oligosaccharides: Gal β 1→4GlcNAc β 1→, Gal β 1→4-(Fuc α 1→3)GlcNAc β 1→, GlcNAc β 1→, Neu5Ac α 2→3-Gal β 1→4GlcNAc β 1 and Neu5Ac α 2→6Gal β 1→4-GlcNAc β 1→ in their outer chains. Whether the structures of the Asn-linked oligosaccharides attached to PrP^c differ significantly from those of PrP^{sc} remains to be determined.

Both mature PrP isoforms have two cysteine residues at codons 179 and 214 that are linked by an intramolecular disulphide bond [155]. This disulphide bond forms a loop which contains both Asn-linked oligosaccharides as well as a variant amino acid at codon 189 found in long-incubation-period mice (*Prn-p^b*).

A 22-amino-acid signal peptide is found at the *N*-terminus of PrP. Like all other signal peptides, it contains a hydrophobic core and a consensus cleavage site [153,169,170]. Cell-free translation studies have demonstrated the cleavage of the PrP signal peptide [171] and *N*-terminal amino acid sequencing of PrP^c and PrP^{sc} confirm the cleavage [116,155,172].

The *C*-terminal hydrophobic segment of both PrP isoforms appears to be removed during maturation [95] and a GPI anchor added [91]. Both PrP^c and PrP^{sc} have GPI anchors. Endopeptidase digestions combined with amino acid analysis and mass-spectrometric studies have shown that the GPI anchor of PrP^{sc} is attached to Ser-231 [173], as previously predicted [174].

Arg-25 and Arg-37 in PrP^{sc} contain an unknown modification which prevents their detection by gas-phase sequencing using the Edman degradation [145,155]. Arg-25 of PrP^c contains a similar modification [155]. Whether Arg-37 also contains this modification is uncertain; however, Arg-48 of PrP^{sc} does not contain the modification.

PROPERTIES OF PRION LIPOSOMES

Search for a scrapie-specific nucleic acid

The transfer of scrapie prion infectivity from rods to liposomes provided a new method by which we could search for a hidden or cryptic nucleic acid within the prion. Treatment of DLPCs as well as liposomes with nucleases or Zn²⁺ failed to alter scrapie infectivity [78]. In control experiments, nucleic acids were added to DLPCs at a concentration of one molecule per infectious unit. Under the conditions of our experiments, nucleases or Zn²⁺ degraded the exogenously added DNA or RNA molecules to < 1 % of their initial concentration. In the experiments described above, nucleases were added after the DLPCs and liposomes were formed. In subsequent studies, the nucleases were present during the formation of the DLPCs [79]. As before, no change in prion titre was observed after prolonged incubations.

Irradiation at 254 nm of the DLPCs produced an inactivation curve virtually identical with that observed for the rods in the experiment reported here and earlier studies (Fig. 5a) [79]. The kinetics of inactivation of scrapie infectivity by u.v. irradiation yielded exponential survival curves characteristic of a single-hit process.

Fig. 5. Inactivation of scrapie prion rods and liposomes by irradiation

Amyloid rods (□) containing $\sim 30 \mu\text{g}/\text{ml}$ of PrP 27-30 or dispersed PrP 27-30 (▲) in PC (20 mg/ml) as DLPCs were prepared as previously described [77,78,94,175]. After irradiation studies, samples were inoculated intracerebrally into Syrian hamsters for scrapie bioassay [74]. $\log(\text{Titre of irradiated samples}/\text{titre of unirradiated samples})$ was plotted as a function of the irradiation dose. Curves were fitted visually. (a) U.v. irradiation of prions. Fractions were irradiated with u.v. light with a 5 GE germicidal lamp for increasing periods of time. (b) Ionizing radiation of prions. All samples were frozen in ethanol/solid CO_2 baths prior to storage at -70°C . The samples were irradiated with 13 MV electrons at -135°C [175,179]. Controls receiving no irradiation were subjected to the same protocol.

From these ultraviolet inactivation curves, we estimated that the D_{37} values for the DLPCs and rods were 17 and $24 \text{ J}/\text{m}^2$ respectively. In another study, we reported a D_{37} value of $17\text{--}22 \text{ kJ}/\text{m}^2$ for purified prion rods [175]. The resistance of scrapie infectivity to inactivation by irradiation at 254 nm suggests that, if prions contain a nucleic acid, it will be approx. 5 bases in length for a single-stranded molecule or 30–45 base-pairs for a double-stranded molecule [175]. The D_{37} values for purified prion rods and DLPCs are in good agreement with values reported two decades earlier for murine scrapie agent in brain homogenates [37].

Prolonged exposure of the rods and DLPCs to proteolytic digestion resulted in a significant decrease in scrapie infectivity [79]. Inactivation of prion rods and DLPCs by proteinase K was both a function of the time of digestion and the concentration of proteinase, as reported earlier for both partially purified [64] and more extensively purified [75] samples.

One explanation for the protease resistance of PrP 27-30 and prion infectivity has been the aggregation of PrP 27-30 into amyloid rods [77]. Dissociation of PrP 27-30 in DLPCs allowed us to test this hypothesis. The kinetics of scrapie infectivity loss as a function of proteinase K digestion were similar for both the DLPCs and rods [79]. After 24 h of digestion at 37°C with $100 \mu\text{g}$ of proteinase K/ml, the prion titre for both DLPCs and rods decreased by $> 10^2 \text{ ID}_{50}$ units/ml; with $500 \mu\text{g}$ of proteinase K/ml, the titre decreased by $> 10^4 \text{ ID}_{50}$ units/ml.

These experiments, as well as studies on immunoaffinity-purified PrP^{Sc}, argue that resistance of PrP 27-30 to enzyme-catalysed proteolysis is an intrinsic property of the scrapie PrP isoform and not merely a consequence of its polymerization into amyloid rods.

Early radiobiological investigations on the scrapie agent suggested that it might have an M_r of $< 150\,000$ and it might not possess a nucleic acid [37,38]. While the results of these studies were often quoted, their implications have been frequently ignored and more recently their interpretation seriously questioned [49,176–178]. Prion rods and liposomes were irradiated with increasing doses up to 400 Mrad. The inactivation curves (Fig. 5b) were virtually identical with those seen with microsomal fractions isolated from scrapie-infected hamster brain before and after extraction with detergent [180]. The survival curves for all forms were exponential over a range of more than 10^5 infectious units, which is characteristic of a single-hit process.

Results from 14 separate experiments with various types of preparations, each irradiated as a frozen solution, yielded a mean target size for the scrapie agent of $55000 \pm 9000 \text{ Da}$ [180]. The early radiation studies [38,181–183], corrected for temperature effects [184,185], yielded target sizes between 60000 and 150000 Da. An equally important finding of our studies is that the target size for scrapie infectivity appears to be independent of the prion form [180]. Samples containing aggregates in the form of amyloid rods yielded an inactivation curve indistinguishable from those curves observed with microsomes, crude brain homogenates or liposomes. Clearly the various forms of the scrapie prion do not influence its ionizing radiation target size.

Although there is no chemical or physical evidence for a scrapie-specific nucleic acid within the prion, reports of scrapie 'strains' or isolates are interpreted by some investigators as evidence for a scrapie agent genome [186–188]. Studies with *Prn-p^a* and *Prn-p^b* mice, which encode PrP^{Sc} allotypes PrP^{Sc}-A with PrP^{Sc}-B differing by two amino acids [83], are forcing a re-evaluation of the significance and molecular basis of scrapie 'strains' [189]. Passaging prions in *Prn-p^a* (NZW and Swiss), *Prn-p^b* (I/Ln) and (NZW $\times$ I/Ln) F_1 mice as well as mice derived in a (NZW $\times$ I/Ln) F_2 cross established that these mice produce prion isolates with different properties which correlate with their *Prn-p* genotype. This is an important point since C57BL and VM mice were used to derive many distinguishable scrapie isolates [186]. C57BL mice

are *Prn-p^a* and VM mice are *Prn-p^b*. Compared with prions from NZW or Swiss mice, a single passage of prions through I/Ln mice dramatically shortened incubation times in recipient I/Ln mice with no further change in a second syngeneic passage. Conversely, inoculum obtained from a single passage in I/Ln mice delayed the onset of clinical scrapie in recipient NZW mice compared with inocula previously passed through NZW and Swiss mice.

The results of experiments with prion isolates derived from *Prn-p^a* and *Prn-p^b* mice appear to reflect an inbred mouse strain barrier defined by the *Prn* genotype [189]. The *Prn-p^a/Prn-p^b* allotype barrier may be analogous to the 'species barrier' described by Pattison [190], where a marked prolongation of the scrapie incubation time is observed upon interspecies passage but the minimum incubation time is achieved upon a single intraspecies passage. Because all species susceptible to scrapie examined, to date, encode distinct prion proteins [83,153,154,161–164], we hypothesized that the PrP genotype may be responsible for the 'species barrier' [5]. In support of this hypothesis are studies with transgenic mice harbouring hamster (Ha) PrP genes, showing scrapie incubation times of ~ 75 days in these Tg mice inoculated with Ha prions similar to those seen with hamsters [85]. In contrast, non-transgenic mice inoculated with Ha prions have incubation times > 500 days.

While prion isolates with true breeding yet different incubation times in the same inbred host are more compelling as *bonafide* 'strains', there are no biochemical or fluctuation test [191] data to equate genesis of these scrapie prion isolates with nucleic acid mutation. Clearly, the molecular basis for genesis of scrapie 'strains' is an unresolved issue.

Multiple forms of prions

The solubilization of prions with phospholipids has helped to clarify the issues concerning the heterogeneity of the infectious particle with respect to size, density and charge. The apparent heterogeneity of prions is well documented and has plagued scrapie research over the last two decades [39]. The ability of prions to form rods, DLPCs and liposomes each having high prion titres provided definitive evidence that prions may exist in multiple forms [78]. The results of such studies argue persuasively that hydrophobic interactions are responsible for the extreme apparent heterogeneity of prions, as suggested over a decade ago [39,139].

Interconversion of the multiple forms of prions depicted in Fig. 6 depends upon the relative levels of detergent and lipid [79]. As described above, both detergent extraction and limited proteolysis of PrP^{Sc} are required for amyloid rod formation. Dispersion of the rods into DLPCs was accomplished by the addition of detergent and phospholipid, while subsequent removal of detergent produced closed liposomes [78]. Removal of the phospholipid resulted in the reformation of rods with full retention of scrapie infectivity [79]. As illustrated, dispersion of microsomal membranes into DLPCs bypassed rod formation; this approach was used for the immunoaffinity purification studies.

The foregoing studies demonstrate that prions may exist in multiple forms and, to date, the only molecule identified in all of these forms is PrP^{Sc}. The rapid interconversion of various prion forms argues, but does not prove, that prions are likely to be composed only of a single type of macromolecule. It is difficult to envision how a highly charged water-soluble macromolecule such

Fig. 6. Interconversion of multiple prion forms

PrP^{Sc} is a membrane-bound protein which upon limited proteolysis produces PrP 27–30. Detergent extraction of membrane-bound PrP 27–30 produces amyloid rods, which can be dispersed into DLPCs by addition of phospholipids. DLPCs can be formed directly from membranes by addition of detergent and phospholipid. Removal of detergents from DLPCs produces closed liposomes. Removal of phospholipid from liposomes by organic solvent extraction (chloroform/methanol) regenerated the rods. All of the prion forms shown here possess high levels of scrapie infectivity [79]. Reproduced, with permission, from *Annu. Rev. Microbiol.* **43**, © 1989 Annual Reviews Inc.

as a nucleic acid could partition with PrP^{Sc} throughout these interconversion steps and at the same time remain so highly protected that it has eluded all attempts at detection.

SIGNIFICANCE OF PRION LIPOSOMES FOR SCRAPIE RESEARCH

The discovery that scrapie infectivity can be dispersed into DLPCs and liposomes represents a significant advance in prion research. Dispersal of prions into liposomes argues convincingly that rod-shaped aggregates of PrP 27–30 are not required for infectivity. The production of prion liposomes allowed for the first time studies designed to dissect the 'apparent heterogeneity' of scrapie infectivity. Excellent evidence has been accumulated which contends that the monomeric prion is considerably smaller than the smallest known virus, in accord with ionizing radiation target size estimates [180]. Furthermore, the ionizing radiation inactivation curves were found to be independent of the form of the prion.

The interconversion of prion rods, DLPCs and liposomes, all with high levels of infectivity (Fig. 6), argues that prions are likely to be composed of a single hydrophobic macromolecule, i.e. PrP^{Sc}. While it is unlikely that prions contain a second macromolecule such as a hydrophilic nucleic acid, further studies are needed to establish whether or not this is the case [192]. All attempts to reveal a putative hidden or cryptic nucleic acid upon transfer of scrapie infectivity in DLPCs have been unsuccessful, to date [78,79].

Many new experimental approaches directed toward understanding the molecular biology of the prion have already evolved from the prion liposome discovery. For example, physical studies designed to identify a putative scrapie-specific nucleic acid in purified prion preparations have utilized the formations of DLPCs to reduce small contaminating polynucleotides by a factor of 10 (N. Meyer, V. Rosenbaum, B. Schmidt, D. Riesner, K. Gilles, C. Mirenda, D. Groth & S. B. Prusiner, unpublished work). It is noteworthy that no scrapie-specific nucleic acid has been identified, to date. In another study, purified prion rods containing PrP 27–30 were radioiodinated and then dispersed into DLPCs. The ¹²⁵I-PrP 27–30 DLPCs were used as a probe to identify binding proteins in homogenates of rodent organs by ligand blotting [193]. Two proteins of 45 kDa and 110 kDa have been identified by this procedure and designated PrP ligands or Plis. Considerable evidence argues that Pli 45 is identical with glial fibrillary acidic protein. Dispersion of PrP 27–30 into DLPCs was necessary for detection of the Pli proteins. Whether Pli 45 or Pli 110 feature in the entry, spread or synthesis of prions remains to be established.

Further studies using cross-linking reagents and PrP^{Sc}-specific antibodies in combination with prion liposomes may yield important new insights into the novel structure of the scrapie prion. Such studies will undoubtedly be performed in concert with PrP transgenes or recombinant expression vectors encoding a variety of prion proteins. Recent investigations have shown that alterations in PrP sequence are genetically linked to changes in susceptibility to prion diseases in both humans and mice [18,83], and transgenic mouse studies provide a new and

exciting avenue of research on the molecular biology and genetics of prion diseases [85].

The significance of the liposome discovery goes beyond arguing persuasively that PrP^{Sc} is a component of the infectious particle. This finding also opens up new approaches in experimental biology that may help us understand the molecular events of central nervous system degeneration.

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REFERENCES

1. Gajdusek, D. C. (1977) *Science* **197**, 943–960
2. Kimberlin, R. H. (ed.) (1976) *Scrapie in the Mouse*, pp. 1–77, Meadowfield Press, Durham
3. Parry, H. B. (ed.) (1983) *Scrapie Disease in Sheep*, 192 pp, Academic Press, New York
4. M'Gowan, J. P. (1914) *Investigation into the Disease of Sheep Called 'Scrapie'*, 116 pp., William Blackwood and Sons, Edinburgh
5. Westaway, D., Carlson, G. A. & Prusiner, S. B. (1989) *Trends Neurosci.* **12**, 221–227
6. Hourrigan, J., Klingsporn, A., Clark, W. W. & de Camp, M. (1979) in *Slow Transmissible Diseases of the Nervous System* (Prusiner, S. B. & Hadlow, W. J., eds.), vol. 1, pp. 331–356, Academic Press, New York
7. Gordon, W. W. (1966) in *Report of Scrapie Seminar*, ARS 91-53, pp. 53–67, U.S. Department of Agriculture, Washington, DC
8. Parry, H. B. (1962) *Heredity* **17**, 75–105
9. Dickinson, A. G., Young, G. B., Stamp, J. T. & Renwick, C. C. (1965) *Heredity* **20**, 485–503
10. Dickinson, A. G. (1976) in *Slow Virus Diseases of Animals and Man* (Kimberlin, R. H., ed.), pp. 209–241, North-Holland, Amsterdam
11. Hartsough, G. R. & Burger, D. (1965) *J. Infect. Dis.* **115**, 387–392
12. Williams, E. S. & Young, S. (1980) *J. Wildl. Dis.* **16**, 89–98
13. Hope, J., Reekie, L. J. D., Hunter, N., Multhaup, G., Beyreuther, K. *et al.* (1988) *Nature (London)* **336**, 390–392
14. Wilesmith, J. W., Wells, G. A. H., Cranwell, M. P. & Ryan, J. B. M. (1988) *Vet. Rec.* **123**, 638–644
15. Gajdusek, D. C., Gibbs, C. J., Jr. & Alpers, M. (1966) *Nature (London)* **209**, 794–796
16. Gibb, C. J., Jr., Gajdusek, D. C., Asher, D. M., Alpers, M. P., Beck, E. *et al.* (1968) *Science* **161**, 388–389
17. Masters, C. L., Gajdusek, D. C. & Gibbs, C. J., Jr. (1981) *Brain* **104**, 559–588
18. Hsiao, K., Baker, H. F., Crow, T. J., Poulter, M., Owen, F. *et al.* (1989) *Nature (London)* **338**, 342–345
19. Prusiner, S. B. (1987) *N. Engl. J. Med.* **317**, 1571–1581
20. Ridley, R. M., Baker, H. F. & Crow, T. J. (1986) *Psychol. Med.* **16**, 199–207
21. Owen, F., Poulter, M., Lofthouse, R., Collinge, J., Crow, T. J. *et al.* (1989) *Lancet* **i**, 51–52

22. Collinge, J., Harding, A. E., Owen, F., Poulter, M., Lofthouse, R. *et al.* (1989) *Lancet* **ii**, 15–17
23. Goldgaber, D., Goldfarb, L. G., Brown, P., Asher, D. M., Brown, W. T. *et al.* (1989) *Exp. Neurol.*, in the press
24. Doh-ura, K., Tateishi, J., Sasaki, H., Kitamoto, T. & Sakaki, Y. (1989) *Biochem. Biophys. Res. Commun.* **163**, 974–979
25. Weissmann, C. (1989) *Nature (London)* **338**, 298–299
26. Hardy, J. (1989) *Trends Neurosci.* **12**, 168–169
27. Prusiner, S. B. (1987) *Annu. Rev. Med.* **38**, 298–381
28. Hope, J. & Kimberlin, R. H. (1987) *Trends Neurosci.* **10**, 149–151
29. Kimberlin, R. H. & Hope, J. (1987) *Trends Genet.* **3**, 117–118
30. Carp, R. I., Kascsak, R. J., Wisniewski, H. M., Merz, P. A., Rubenstein, R. *et al.* (1989) *Alzheimer Dis. Assoc. Disord.* **3**, 79–99
31. Manuelidis, E. E. & Manuelidis, L. (1989) *Alzheimer Dis. Assoc. Disord.* **3**, 100–109
32. Diener, T. O. (1987) *Cell* **49**, 719–721
33. Prusiner, S. B. & McKinley, M. P. (eds.) (1987) *Prions — Novel Infectious Pathogens Causing Scrapie and Creutzfeldt-Jakob Disease*, 534 pp., Academic Press, Orlando
34. Bock, G. & Marsh, J. (eds.) (1988) *Ciba Found. Symp.* **135**, 1–277
35. Gordon, W. S. (1946) *Vet. Rec.* **58**, 516–520
36. Chandler, R. L. (1961) *Lancet* **i**, 1378–1379
37. Alper, T., Cramp, W. A., Haig, D. A. & Clarke, M. C. (1967) *Nature (London)* **214**, 764–766
38. Alper, T., Haig, D. A. & Clarke, M. C. (1966) *Biochem. Biophys. Res. Commun.* **22**, 278–284
39. Prusiner, S. B., Garfin, D. E., Baringer, J. R., Cochran, S. P., Hadlow, W. J. *et al.* (1979) in *Slow Transmissible Diseases of the Nervous System* (Prusiner, S. B. & Hadlow, H. J., eds.), vol. 2, pp. 425–464, Academic Press, New York
40. Gibbons, R. A. & Hunter, G. D. (1967) *Nature (London)* **215**, 1041–1043
41. Hunter, G. D., Kimberlin, R. H. & Gibbons, R. A. (1968) *J. Theor. Biol.* **20**, 355–357
42. Diener, T. O. (1972) *Nature (London)* **235**, 218–219
43. Field, E. J. (1966) *Br. Med. J.* **2**, 564–565
44. Field, E. J. (1967) *Dtsch. Z. Nervenheilkd.* **192**, 265–274
45. Griffith, J. S. (1967) *Nature (London)* **215**, 1043–1044
46. Pattison, I. H. & Jones, K. M. (1967) *Vet. Rec.* **80**, 1–8
47. Lewin, P. (1972) *Lancet* **i**, 748
48. Lewin, P. (1981) *Can. Med. Assoc. J.* **124**, 1436–1437
49. Rohwer, R. G. (1984) *Nature (London)* **308**, 658–662
50. Adams, D. H. & Field, E. J. (1968) *Lancet* **ii**, 714–716
51. Adams, D. H. (1970) *Pathol. Biol.* **18**, 559–577
52. Rohwer, R. G. (1984) *Science* **223**, 600–602
53. Dickinson, A. G. & Outram, G. W. (1979) in *Slow Transmissible Diseases of the Nervous System* (Prusiner, S. B. & Hadlow, W. J., eds.), vol. 2, pp. 13–31, Academic Press, New York
54. Pattison, I. H. (1988) *Vet. Rec.* **123**, 661–666
55. Gajdusek, D. C. (1986) *UCLA Forum Med. Sci.* **27**, 33–54
56. Gajdusek, D. C. (1988) *J. Neuroimmunol.* **20**, 95–110
57. Marsh, R. F., Malone, T. G., Semancik, J. S., Lancaster, W. D. & Hanson, R. P. (1978) *Nature (London)* **275**, 146–147
58. Malone, T. G., Marsh, R. F., Hanson, R. P. & Semancik, J. S. (1979) *Nature (London)* **278**, 575–576
59. Prusiner, S. B., Groth, D. F., Bildstein, C., Masiarz, F. R., McKinley, M. P. *et al.* (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2984–2988
60. Dickinson, A. G. & Outram, G. W. (1988) *Ciba Found. Symp.* **135**, 63–83
61. Cho, H. J. (1980) *Intervirology* **14**, 213–216
62. Hunter, G. D. & Millson, G. C. (1967) *J. Comp. Pathol.* **77**, 301–337
63. Millson, G. C., Hunter, G. D. & Kimberlin, R. H. (1976) in *Slow Virus Diseases of Animals and Man* (Kimberlin, R. H., ed.), pp. 243–266, American Elsevier, New York
64. Prusiner, S. B., McKinley, M. P., Groth, D. F., Bowman, K. A., Mock, N. I. *et al.* (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6675–6679
65. Prusiner, S. B. (1982) *Science* **216**, 136–144
66. Diener, T. O., McKinley, M. P. & Prusiner, S. B. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5220–5224
67. Carp, R. I., Merz, P. A., Kascsak, R. J., Merz, G. S. & Wisniewski, H. M. (1985) *J. Gen. Virol.* **66**, 1357–1368
68. Hope, J. & Kimberlin, R. H. (1987) *Trends Neurosci.* **10**, 149–151
69. Chamberlin, T. C. (1890) *Science (Old Ser.)* **15**, 92–97 [Reprinted in *Science* (1965) **148**, 754–759]
70. Prusiner, S. B. (1989) *Annu. Rev. Microbiol.* **43**, 345–374
71. Bolton, D. C., McKinley, M. P. & Prusiner, S. B. (1982) *Science* **218**, 1309–1311
72. Prusiner, S. B., Bolton, D. C., Groth, D. F., Bowman, K. A., Cochran, S. P. *et al.* (1982) *Biochemistry* **21**, 6942–6950
73. Prusiner, S. B., Groth, D. F., Cochran, S. P., Masiarz, F. R., McKinley, M. P. *et al.* (1980) *Biochemistry* **19**, 4883–4891
74. Prusiner, S. B., Cochran, S. P., Growth, D. F., Downey, D. E., Bowman, K. A. & Martinez, H. M. (1982) *Ann. Neurol.* **11**, 353–358
75. McKinley, M. P., Bolton, D. C. & Prusiner, S. B. (1983) *Cell* **35**, 57–62
76. Bolton, D. C., McKinley, M. P. & Prusiner, S. B. (1984) *Biochemistry* **23**, 5898–5905
77. Prusiner, S. B., McKinley, M. P., Bowman, K. A., Bolton, D. C., Bendheim, P. E. *et al.* (1983) *Cell* **35**, 349–358
78. Gabizon, R., McKinley, M. P. & Prusiner, S. B. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4017–4021
79. Gabizon, R., McKinley, M. P., Groth, D. F., Kenaga, L. & Prusiner, S. B. (1988) *J. Biol. Chem.* **263**, 4950–4955
80. Gabizon, R., McKinley, M. P., Groth, D. F. & Prusiner, S. B. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6617–6621
81. Carlson, G. A., Kingsbury, D. T., Goodman, P., Coleman, S., Marshall, S. T. *et al.* (1986) *Cell* **46**, 503–511
82. Hunter, N., Hope, J., McConnell, I. & Dickinson, A. G. (1987) *J. Gen. Virol.* **68**, 2711–2716
83. Westaway, D., Goodman, P., Mirenda, C., McKinley, M. P., Carlson, G. *et al.* (1987) *Cell* **51**, 651–662
84. Hsiao, K. K., Westaway, D. A. & Prusiner, S. B. (1988) *Am. J. Hum. Genet.* **43**, A87
85. Scott, M., Foster, D., Mirenda, C., Serban, D., Coufal, F. *et al.* (1989) *Cell* **59**, 847–857
86. Kitamoto, T., Tateishi, J., Tashima, T., Takeshita, I., Barry, R. A. *et al.* (1986) *Ann. Neurol.* **20**, 204–208
87. Roberts, G. W., Lofthouse, R., Brown, R., Crow, T. J., Barry, R. A. *et al.* (1986) *N. Engl. J. Med.* **315**, 1231–1233
88. DeArmond, S. J., McKinley, M. P., Barry, R. A., Braunfeld, M. B., McColloch, J. R. *et al.* (1985) *Cell* **41**, 221–235
89. Serban, D., Taraboulos, A., DeArmond, S. J. & Prusiner, S. B. (1990) *Neurology*, in the press
90. Butler, D. A., Scott, M. R. D., Bockman, J. M., Borchelt, D. R., Taraboulos, A. *et al.* (1988) *J. Virol.* **62**, 1558–1564
91. Stahl, N., Borchelt, D. R., Hsiao, K. K. & Prusiner, S. B. (1987) *Cell* **51**, 229–240
92. Taraboulos, A., Serban, D. & Prusiner, S. B. (1989) *J. Cell Biol.* **109**, 224a (abstr.)

93. Borchelt, D. R., Scott, M., Taraboulos, A., Stahl, N. & Prusiner, S. B. (1990) *J. Cell Biol.*, in the press
94. Barry, R. A. & Prusiner, S. B. (1986) *J. Infect. Dis.* **154**, 518–521
95. Oesch, B., Westaway, D., Wälchli, M., McKinley, M. P., Kent, S. B. H. *et al.* (1985) *Cell* **40**, 735–746
96. Barry, R. A., McKinley, M. P., Bendheim, P. E., Lewis, G. K., DeArmond, S. J. *et al.* (1985) *J. Immunol.* **135**, 603–613
97. McKinley, M. P., Braunfeld, M. B., Bellinger, C. G. & Prusiner, S. B. (1986) *J. Infect. Dis.* **154**, 110–120
98. Meyer, R. K., McKinley, M. P., Bowman, K. A., Barry, R. A. & Prusiner, S. B. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2310–2314
99. Barry, R. A. & Prusiner, S. B. (1987) in *Prions – Novel Infectious Pathogens Causing Scrapie and Creutzfeldt–Jakob Disease* (Prusiner, S. B. & McKinley, M. P., eds.), pp. 239–275, Academic Press, Orlando
100. Ito, M., Shinagawa, M., Doi, S., Sasaki, S., Isomura, H. *et al.* (1988) *Microbiol. Immunol.* **32**, 749–753
101. Bockman, J. M., Kingsbury, D. T., McKinley, M. P., Bendheim, P. E. & Prusiner, S. B. (1985) *N. Engl. J. Med.* **312**, 73–78
102. Bockman, J. M., Prusiner, S. B., Tateishi, J. & Kingsbury, D. T. (1987) *Ann. Neurol.* **21**, 589–595
103. Brown, P., Coker-Vann, M., Pomeroy, K., Franko, M., Asher, D. M. *et al.* (1986) *N. Engl. J. Med.* **314**, 547–551
104. Klatzo, I., Gajdusek, D. C. & Zigas, V. (1959) *Lab. Invest.* **8**, 799–847
105. Beck, E., Daniel, P. M. & Parry, H. B. (1964) *Brain* **87**, 153–176
106. Bruce, M. E. & Fraser, H. (1975) *Neuropathol. Appl. Neurobiol.* **1**, 189–202
107. Wisniewski, H. M., Bruce, M. E. & Fraser, H. (1975) *Science* **190**, 1108–1110
108. Bahmanyar, S., Williams, E. S., Johnson, F. B., Young, S. & Gajdusek, D. C. (1985) *J. Comp. Pathol.* **95**, 1–5
109. Fraser, H. & Bruce, M. E. (1983) *EMBO J.* **59**, 281–290
110. Bendheim, P. E., Barry, R. A., DeArmond, S. J., Stites, D. P. & Prusiner, S. B. (1984) *Nature (London)* **310**, 418–421
111. Kingsbury, D. T., Smeltzer, D. & Bockman, J. (1984) *Int. Congr. Virol.* 6th, Sendai, Japan, p. 70 (abstr.)
112. Race, R. E., Fadness, L. H. & Chesebro, B. (1987) *J. Gen. Virol.* **68**, 1391–1399
113. Caughey, B., Race, R. E., Ernst, D., Buchmeier, M. J. & Chesebro, B. (1989) *J. Virol.* **63**, 175–181
114. Gibbs, C. J., Jr., Joy, A., Heffner, R., Franko, M., Miyazaki, M. *et al.* (1985) *N. Engl. J. Med.* **313**, 734–738
115. Diring, H., Gelderblom, H., Hilmert, H., Ozel, M., Edelbluth, C. *et al.* (1983) *Nature (London)* **306**, 476–478
116. Hope, J., Morton, L. J. D., Farquhar, C. F., Multhaup, G., Beyreuther, K. *et al.* (1986) *EMBO J.* **5**, 2591–2597
117. Manuelidis, L., Sklaviadis, T. & Manuelidis, E. E. (1987) *EMBO J.* **6**, 341–347
118. Braig, H. & Diring, H. (1985) *EMBO J.* **4**, 2309–2312
119. Czub, M., Braig, H. R. & Diring, H. (1988) *J. Gen. Virol.* **69**, 1753–1756
120. Chesebro, B., Race, R., Wehrly, K., Nishio, J., Bloom, M. *et al.* (1985) *Nature (London)* **315**, 331–333
121. Czub, M., Braig, H. R., Blode, H. & Diring, H. (1986) *Arch. Virol.* **91**, 383–386
122. McKinley, M. P., Butler, D. A. & Prusiner, S. B. (1987) *J. Cell Biol.* **105**, 317a (abstr.)
123. Rubenstein, R., Kascsak, R. J., Merz, P. A., Papini, M. C., Carp, R. I. *et al.* (1986) *J. Gen. Virol.* **67**, 671–681
124. Shinagawa, M., Muneata, E., Doi, S., Takahashi, K., Goto, H. *et al.* (1986) *J. Gen. Virol.* **67**, 1745–1750
125. Hunter, G. G. (1979) in *Slow Transmissible Diseases of the Nervous System* (Prusiner, S. B. & Hadlow, W. J., eds.), vol. 2, pp. 365–385, Academic Press, New York
126. Milson, G. C. & Manning, E. J. (1979) in *Slow Transmissible Diseases of the Nervous System* (Prusiner, S. B. & Hadlow, W. J., eds.), vol. 2, pp. 409–424, Academic Press, New York
127. Hunter, G. D. (1972) *J. Infect. Dis.* **125**, 427–440
128. Kimberlin, R. H., Millson, G. C. & Hunter, G. D. (1971) *J. Comp. Pathol.* **81**, 383–391
129. Millson, G., Hunter, G. D. & Kimberlin, R. H. (1971) *J. Comp. Pathol.* **81**, 255–265
130. Semancik, J. S., Marsh, R. F., Geelen, J. L. & Hanson, R. P. (1976) *J. Virol.* **18**, 693–700
131. Malone, T. G., Marsh, R. F., Hanson, R. P. & Semancik, J. S. (1978) *J. Virol.* **25**, 933–935
132. Liao, T. H. (1975) *J. Biol. Chem.* **250**, 3831–3836
133. Cho, H. J. & Greig, A. S. (1975) *Nature (London)* **257**, 685–686
134. Cho, H. J. (1976) *Nature (London)* **262**, 411–412
135. Cho, H. J., Greig, A. S., Corp, C. R., Kimberlin, R. H., Chandler, R. L. *et al.* (1977) *Nature (London)* **267**, 459–460
136. Siakotos, A. N., Gajdusek, D. C., Gibbs, C. J., Jr., Traub, R. D. & Bucana, C. (1976) *Virology* **70**, 230–237
137. Brown, P., Green, E. M. & Gajdusek, D. C. (1978) *Proc. Soc. Exp. Biol. Med.* **158**, 513–516
138. Prusiner, S. B., Hadlow, W. J., Eklund, C. M. & Race, R. E. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4656–4660
139. Prusiner, S. B., Hadlow, W. J., Garfin, D. E., Cochran, S. P., Baringer, J. R. *et al.* (1978) *Biochemistry* **17**, 4993–4997
140. Marsh, R. F. & Kimberlin, R. H. (1975) *J. Infect. Dis.* **131**, 104–110
141. Kimberlin, R. & Walker, C. (1977) *J. Gen. Virol.* **34**, 295–304
142. Kimberlin, R. H. & Walker, C. A. (1978) *J. Gen. Virol.* **39**, 487–496
143. McKinley, M. P., Meyer, R., Kenaga, L., Rahbar, F., Serban, A. *et al.* (1988) *J. Cell Biol.* **107**, 725a
144. Somerville, R. A., Ritchie, L. A. & Gibson, P. H. (1989) *J. Gen. Virol.* **70**, 25–35
145. Hope, J., Multhaup, G., Reekie, L. J. D., Kimberlin, R. H. & Beyreuther, K. (1988) *Eur. J. Biochem.* **172**, 271–277
146. Glenner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120**, 885–890
147. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L. *et al.* (1987) *Nature (London)* **325**, 733–736
148. Prusiner, S. B., Groth, D. F., McKinley, M. P., Cochran, S. P., Bowman, K. A. & Kasper, K. C. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4606–4610
149. Sklaviadis, T. K., Manuelidis, L. & Manuelidis, E. E. (1989) *J. Virol.* **63**, 1212–1222
150. Judd, A., Williamson, J. L. & Marsh, R. F. (1989) *J. Virol.* **43**, 1683–1694
151. Prusiner, S. B., Groth, D. F., Bolton, D. C., Kent, S. B. & Hood, L. E. (1984) *Cell* **38**, 127–134
152. Kretzschmar, H. A., Prusiner, S. B., Stowring, L. E. & DeArmond, S. J. (1986) *Am. J. Pathol.* **122**, 1–5
153. Basler, K., Oesch, B., Scott, M., Westaway, D., Wälchli, M. *et al.* (1986) *Cell* **46**, 417–428
154. Locht, C., Chesebro, B., Race, R. & Keith, J. M. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6372–6376
155. Turk, E., Teplow, D. B., Hood, L. E. & Prusiner, S. B. (1988) *Eur. J. Biochem.* **176**, 21–30
156. Chen, S.-H., Habib, G., Yang, C.-Y., Gu, Z.-W., Lee, B. R. *et al.* (1987) *Science* **238**, 363–366
157. Feagin, J. E., Abraham, J. M. & Stuart, K. (1988) *Cell* **53**, 413–422

158. Powell, L. M., Wallis, S. C., Pease, R. J., Edwards, Y. H., Knott, T. J. *et al.* (1987) *Cell* **50**, 831–840
159. Shaw, J. M., Feagin, J. E., Stuart, K. & Simpson, L. (1988) *Cell* **53**, 401–411
160. Sparkes, R. S., Simon, M., Cohn, V. H., Fournier, R. E. K., Lem, J. *et al.* (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7358–7362
161. Kretzschmar, H. A., Stowring, L. E., Westaway, D., Stubblebine, W. H., Prusiner, S. B. *et al.* (1986) *DNA* **5**, 315–324
162. Lowenstein, D. H., Butler, D. A., Westaway, D., McKinley, M. P., DeArmond, S. J. & Prusiner, S. B. (1990) *Mol. Cell. Biol.*, in the press
163. Liao, Y.-C., Tokes, Z., Lim, E., Lackey, A., Woo, C. H. *et al.* (1987) *Lab. Invest.* **57**, 370–374
164. Goldman, W., Hunter, N., Multhaup, G., Salbalm, J. M., Foster, J. D. *et al.* (1988) *Alzheimer Dis. Assoc. Disord.* **2** (Suppl.), 331 (abstr.)
165. Westaway, D. & Prusiner, S. B. (1986) *Nucleic Acids Res.* **14**, 2035–2044
166. Bolton, D. C., Meyer, R. K. & Prusiner, S. B. (1985) *J. Virol.* **53**, 596–606
167. Haraguchi, T., Fisher, S., Olofsson, S., Endo, T., Groth, D. *et al.* (1989) *Arch. Biochem. Biophys.* **274**, 1–13
168. Endo, T., Groth, D., Prusiner, S. B. & Kobata, A. (1989) *Biochemistry* **28**, 8380–8388
169. Robakis, N. K., Sawh, P. R., Wolfe, G. C., Rubenstein, R., Carp, R. I. *et al.* (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6377–6381
170. Bazan, J. F., Fletterick, R. J., McKinley, M. P. & Prusiner, S. B. (1987) *Protein Eng.* **1**, 125–135
171. Hay, B., Barry, R. A., Lieberburg, I., Prusiner, S. B. & Lingappa, V. R. (1987) *Mol. Cell. Biol.* **7**, 914–920
172. Bolton, D. C., Bendheim, P. E., Marmorstein, A. D. & Potempska, A. (1987) *Arch. Biochem. Biophys.* **258**, 579–590
173. Stahl, N. & Prusiner, S. B. (1989) *Proc. Symp. Protein Soc.* 3rd, Seattle, S160 (abstr.)
174. Ferguson, M. A. J. & Williams, A. F. (1988) *Annu. Rev. Biochem.* **57**, 285–320
175. Bellinger-Kawahara, C., Cleaver, J. E., Diener, T. O. & Prusiner, S. B. (1987) *J. Virol.* **61**, 159–166
176. Rohwer, R. G. & Gajdusek, D. C. (1980) in *Search for the Cause of Multiple Sclerosis and Other Chronic Diseases of the Central Nervous System* (Boese, A., ed.), pp. 333–355, Verlag Chemie, Weinheim
177. Alper, T. (1985) *Nature (London)* **317**, 750
178. Rohwer, R. G. (1986) *Nature (London)* **320**, 381
179. Harmon, J. T., Nielsen, T. B. & Kempner, E. S. (1985) *Methods Enzymol.* **117**, 65–94
180. Bellinger-Kawahara, C. G., Kempner, E., Groth, D. F., Gabizon, R. & Prusiner, S. B. (1988) *Virology* **164**, 537–541
181. Field, E. J., Farmer, F., Caspary, E. A. & Joyce, G. (1969) *Nature (London)* **222**, 90–91
182. Latarjet, R. (1979) in *Slow Transmissible Diseases of the Nervous System* (Prusiner, S. B. & Hadlow, W. J., eds.), vol. 2, pp. 387–408, Academic Press, New York
183. Gibbs, C. J., Jr., Gajdusek, D. C. & Latarjet, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6268–6270
184. Kempner, E. S., Miller, J. H. & McCreery, M. J. (1986) *Anal. Biochem.* **156**, 140–146
185. Kempner, E. S., Wood, R. & Salovey, R. (1986) *J. Polym. Sci. Polym. Phys. Ed.* **24**, 2337–2343
186. Bruce, M. E. & Dickinson, A. G. (1987) *J. Gen. Virol.* **68**, 79–89
187. Kimberlin, R. H., Cole, S. & Walker, C. A. (1987) *J. Gen. Virol.* **68**, 1875–1881
188. Kimberlin, R. H., Walker, C. A. & Fraser, H. (1989) *J. Gen. Virol.* **70**, 2017–2025
189. Carlson, G. A., Westaway, D., DeArmond, S. J., Peterson-Torchia, M. & Prusiner, S. B. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7475–7479
190. Pattison, I. H. (1965) in *Slow, Latent and Temperate Virus Infections*, NINDB Monogr. 2 (Gajdusek, D. C., Gibbs, C. J., Jr. & Alpers, M. P., eds.), pp. 249–257, U.S. Government Printing Office, Washington, DC
191. Luria, S. E. & Delbruck, M. (1943) *Genetics* **28**, 491–511
192. Weissmann, C. (1989) *Nature (London)* **338**, 298–299
193. Oesch, B. & Prusiner, S. B. (1989) *Clin. Res.* **37**, 566A (abstr.)