Folia
Neuropathologica

# Cell death and autophagy in prion diseases (transmissible spongiform encephalopathies)

**Paweł P. Liberski[1], David R. Brown[2], Beata Sikorska[1], Byron Caughey[3], Paul Brown[4]**

[1]Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, Poland; [2]Department of Biology and Biochemistry, University of Bath, Bath, UK; [3]Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA; [4]retired, USA

### Abstract

*Neuronal autophagy, like apoptosis, is one of the mechanisms of programmed cell death. In this review, we summarize current information about autophagy in naturally occurring and experimentally induced scrapie, Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker syndrome against the broad background of neural degenerations in transmissible spongiform encephalopathies (TSEs). Typically a sequence of events is observed: from a part of the neuronal cytoplasm sequestrated by concentric arrays of double membranes (phagophores); through the enclosure of the cytoplasm and membrane proliferation; to a final transformation of the large area of the cytoplasm into a collection of autophagic vacuoles of different sizes. These autophagic vacuoles form not only in neuronal perikarya but also in neurites and synapses. On the basis of ultrastructural studies, we suggest that autophagy may play a major role in transmissible spongiform encephalopathies and may even participate in the formation of spongiform change.*

*Key words: autophagy, apoptosis, prion diseases, neurons, ultrastructure.*

## Introduction

The transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of neuro-degenerative disorders which include kuru (Fig. 1) [73,89,135], Creutzfeldt-Jakob disease (CJD) [76], Gerstmann-Sträussler-Scheinker (GSS) disease [157], and fatal familial insomnia (FFI) in humans [147], natural scrapie in sheep (Fig. 2), goats [53,54,57], and mouflons [224], transmissible mink encephalopathy (TME) in ranch-reared mink [35], chronic wasting disease (CWD) of mule deer and elk in North America [137,221,222], bovine spongiform encephalopathy (BSE) or "mad cow disease" [218], and its analogues in several exotic species of antelopes [55,68,106,115] and wild felids in zoological gardens [223], and feline spongiform encephalopathy (FSE) in domestic cats [226].

The cause of these disorders is still not completely understood. Despite a wide acceptance for the prion

**Communicating author:**

Prof. Paweł P. Liberski, Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, Czechosłowacka Str. 8/10, PL 92-216 Lodz, Poland, tel./fax: +48 42 679 14 77, Email: ppliber@csk.am.lodz.pl

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown

**Fig. 1.** Kuru-affected children. Courtesy of Dr Carleton Gajdusek, Amsterdam, the Netherlands



**Fig. 2.** Scrapie in a Romanov sheep. Courtesy of Prof. Jeanne Brugere- Picoux, Paris, France

theory, the name of the infectious agent (a "prion") still reflects our ignorance or at the very least, our uncertainties of its nature [44,139,152]. Those who prefer to view this pathogen as composed predominantly or exclusively of a pathologically misfolded protein use the term "prion" [184]; hence the term "prion diseases" [67,217]. In this review, we will use the term "$PrP^c$" to designate the normal soluble and proteinase K-sensitive cellular protein, and the term "$PrP^{TSE}$" to designate all of its pathological forms, including intermediate species that are sensitive to proteinase K (PK) digestion as well as the mature amyloid species that is insoluble and partially PK-resistant [32].

Other hypotheses, albeit less widely accepted, are still not formally rejected. The "virino" hypothesis suggests that the pathogen is a molecular chimera composed of a yet-to-be-discovered nucleic acid and a shell-protein, which is host-encoded (could be PrP) [113]. The fact that RNA enhances conversion of $PrP^c$ into $PrP^{TSE}$ *in vitro* is interesting in this context [56]. The "unified theory" of Weissmann [216], not unlike the virino theory, suggests that the agent is a molecular chimera in which $PrP^{TSE}$ confers infectivity and an unidentified oligonucleotide specifies strain characteristics. The virus hypothesis simply suggests that the pathogen is a yet-to-be-identified unconventional virus [58,72,216]. To this end, virus-like particles have been repeatedly shown in all TSEs studied so far [140,144,153].

## PrP, the PrP gene, the "prion hypothesis" and strains of the pathogen

$PrP^c$ is a highly conserved sialoglycoprotein encoded by a cellular gene mapped to chromosome 20 in man (*Prion Protein*; *PRNP*) and chromosome 2 in mouse (Fig. 3) [7,16,45,90,176,202,204]. The gene is ubiquitous [193,194,225]; it has been cloned from numerous mammalian species, included marsupials, and there are analogues of this gene in birds [71,93], reptiles [200], amphibians [209], and even fish [177].

The molecular biology of PrP has been extensively studied. Human $PrP^c$ contains 253 amino acids encoded by an intronless open reading frame (ORF) (Fig. 3) [7]. As a result, alternative splicing does not occur. Three forms of $PrP^c$ exist – one completely translocated and two transmembrane variants, $^{Ctm}PrP$ and $^{Ntm}PrP$ [94,95,214] (Fig. 4) – and the sequence encoding residues 151-165 that form the transmembrane region is highly conserved [214]. Furthermore, $PrP^c$ undergoes endoproteolytic cleavage to yield 17 kDa N-terminally truncated form C1, while $PrP^{TSE}$ yields a slightly larger peptide designated C2 (Fig. 3) [43].

The "prion" hypothesis, which is deeply rooted in the association between $PrP^{TSE}$ and infectivity, was formulated by Stanley B. Prusiner in 1982 [10,59,161,181,182]. The hypothesis postulated that the scrapie agent was a proteinaceous infectious particle, because infectivity was dependent on protein but resistant to methods known to inactivate nucleic acids. A similar proposal was presented a decade earlier by Gibbons and Hunter [75], Griffith [85], and Levine [130], who all expanded the earlier suggestion of Alper and her co-workers [1], based on irradiation studies, that scrapie agent was devoid of disease-specific nucleic acid. Furthermore, other investigators in the late 1970s and early 1980s had found that scrapie infectivity was sensitive to proteolytic digestion [47,166].
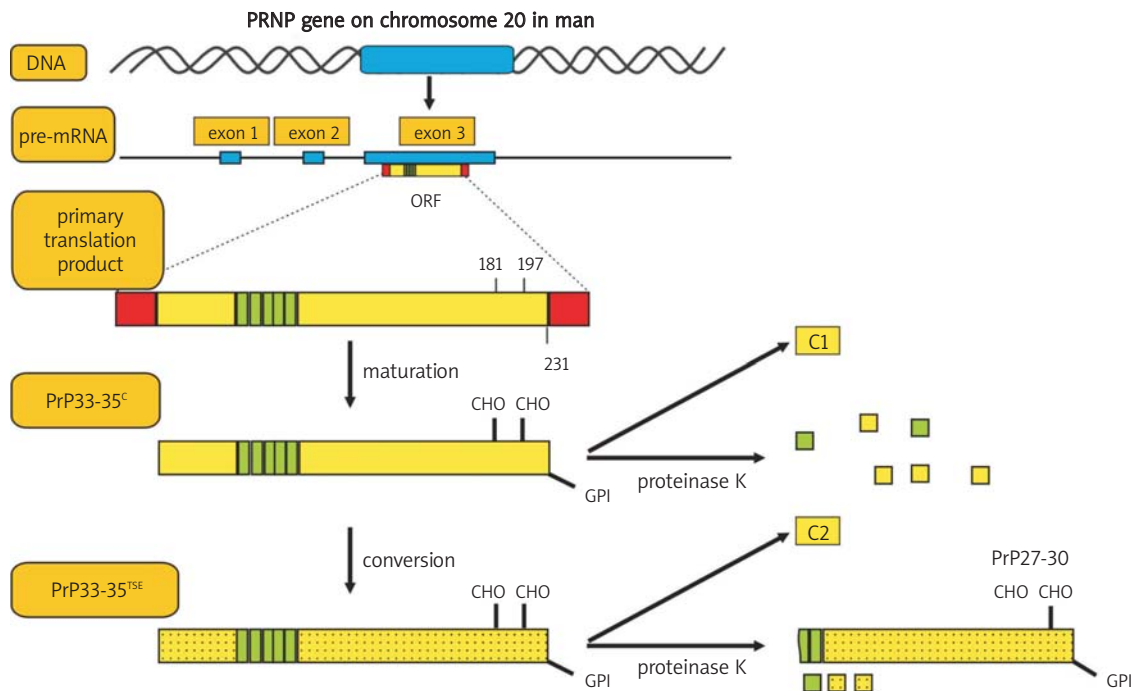
**Fig. 3.** Scheme of PrP generation. The *PRNP* gene (the name *PRP* was already used for Prolyine Rich Protein) is located on chromosome 20. There are 2 exons in humans (in some other species such as mice and cattle, three) and the whole open reading frame (ORF) is located within exon 2 (or 3 in 3-exon species). During the maturation process two glycans (CHO) at positions 181 and 197 are added. The final product is a cellular isoform of PrP (PrP$^c$) which is converted into pathological isoform (PrP$^{TSE}$). PrP$^c$ undergoes endoproteolytic cleavage (red arrows) to yield 17 kDa N-terminally truncated form C1 while PrP$^{TSE}$ yields a slightly larger peptide designated C2. Following proteinase treatment (black arrows), PrP$^c$ is completely degraded whereas PrP$^{TSE}$ is only partially degraded and yields a "core protein" designated PrP 27-30.

The PrP species found to copurify with scrapie infectivity, PrP 27-30, is a proteolytic cleavage product of pathologically misfolded protein, PrP$^{TSE}$. However, PrP$^{TSE}$ is not the *primary* product of the cellular gene, which encodes both PrP$^c$ and PrP$^{TSE}$ (Fig. 3). The amino acid sequences and posttranslational modifications, i.e. glycosylation and a glycophosphatidylinositol (GPI) anchor, of PrP$^c$ and PrP$^{TSE}$ are identical, but their physico-chemical characteristics are strikingly different [207,208]. In particular, PrP$^c$ is completely degraded by limited proteolysis, but PrP$^{TSE}$ is only partially degraded, yielding a core protein (PrP 27-30), which may be visualised by electron microscopy as amyloid-like fibrillar structures called scrapie-associated fibrils (SAF) (Fig. 5) [163,164], currently better known as prion rods [185].
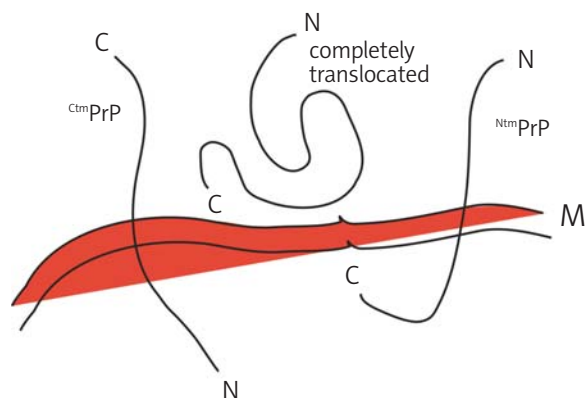


**Fig. 4.** Three different forms of PrP. Completely translocated and two transmembrane variants – $^{Ctm}$PrP and $^{Ntm}$PrP. C – C-terminal, N – N-terminal, M – membrane

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown

## Cellular trafficking of PrP$^c$ and PrP$^{TSE}$

PrP$^c$, as is true of all cell surface glycoproteins, is synthesized first in the endoplasmic reticulum, matured in the Golgi apparatus, and transported in membrane-bound vesicles to the plasma membrane, where it is usually anchored by the GPI moiety (Fig. 6). Once it is on the cell surface, PrP$^c$ can be subjected to endocytosis and either degraded in lysosomes or recycled to the plasma membrane via recycling endosomes [92,149,150,180,198]. In peripheral (PNS) and central nervous system (CNS) neurons fast anterograde and retrograde axonal transport of PrP$^c$ have also been detected [11,170,171], facilitating movement to and from neural extremities.

Coincident with becoming infected, cells can bind and internalize exogenous PrP$^{TSE}$ aggregates [97,98]. Depending on cell type, PrP$^{TSE}$ internalization can involve heparan sulphate [97,98], laminin receptor [169], and/or ferritin transporters [167]. However, the presence of PrP$^c$ does not seem to be required as a receptor for exogenous PrP$^{TSE}$ [97,149].
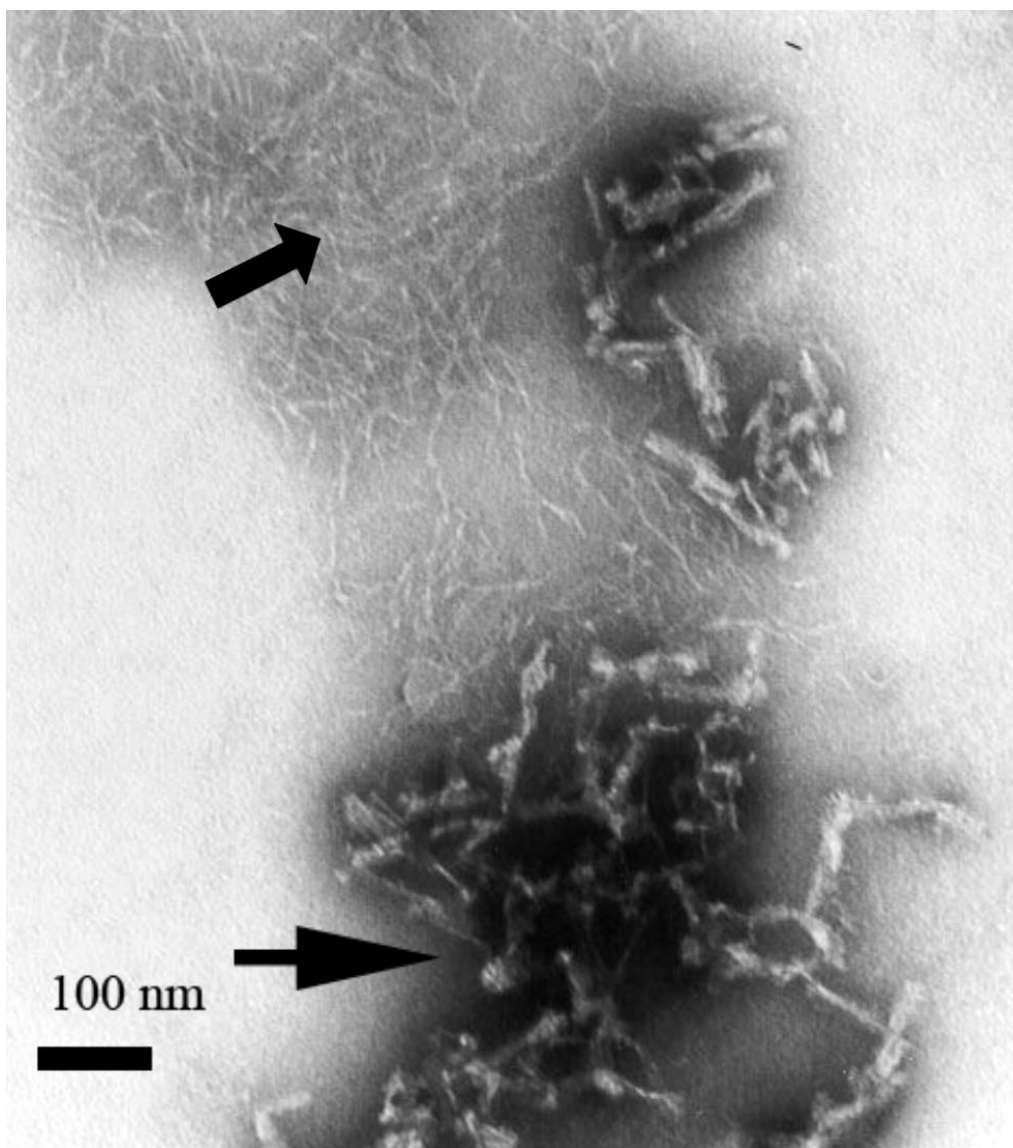


**Fig. 5.** Scrapie-associated fibrils (lower arrow) visualized by negative-staining electron microscopy. An upper arrow points to protofilaments. Original magn. × 30 000; bar = 100 nm
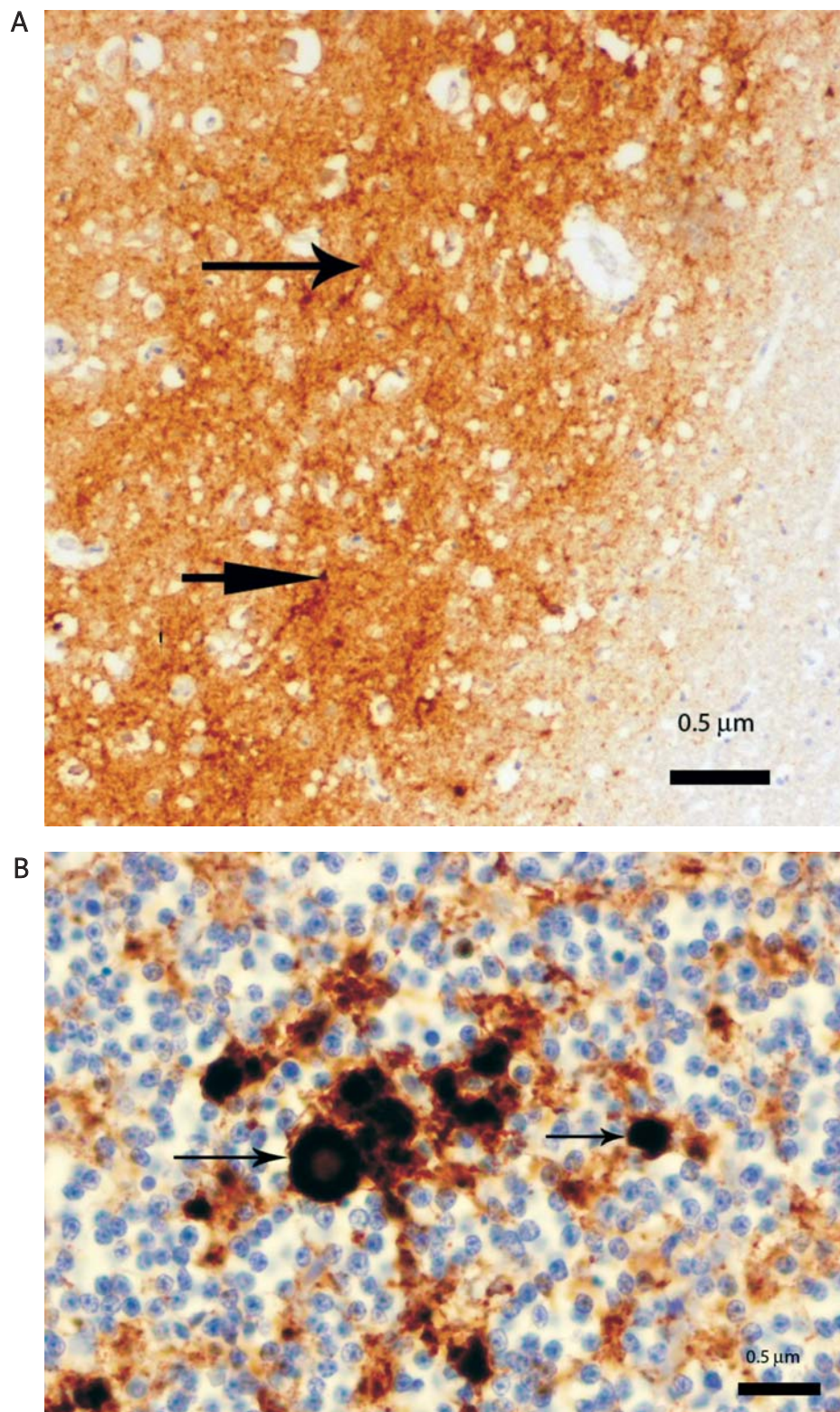
Once cells are infected with a TSE agent *in vitro*, new PrP[TSE] formation appears to occur on the plasma membrane and/or along an endocytic pathway to lysosomes [12,13,41,42]. However, in cells expressing familial TSE-associated PrP[c] mutants, the acquisition of PrP[TSE]-like properties can begin to occur spontaneously at the earliest stages of PrP biosynthesis [92]. In TSE-infected animals, it appears that PrP[TSE] can also be formed at extracellular sites. This is especially likely in scrapie-infected transgenic mice that express only anchorless PrP[c] that is secreted from cells rather than bound to membranes by the GPI moiety [46]. In these mice, PrP[TSE] accumulates almost exclusively in large extracellular amyloid plaques, which are the likely sites of conversion to PrP[TSE].

Because the spread of TSE infections and PrP[TSE] from peripheral sites to the central nervous system follows neuroanatomical pathways [114,159, for review, see ref. 81], it is important to understand the neuronal transport mechanisms. Axonal transport processes, but not the fast form, appear to be involved [5,6,118,125,186]. However, the precise mechanisms by which PrP[TSE] is transported between and within neural cells have not been well defined *in vivo*. Recent studies in cultured neuronal cells have shown that endocytosed exogenous PrP[TSE] can be found in Rab7-positive late endosomes and transported in acidic vesicles along neurites to points of contact with other cells [149]. Other *in vitro* studies have provided evidence that PrP[TSE] can be released from cells [195] in infectious particles that are vesicular and exosome-like (Fig. 7) [3,64,195]. Membrane-associated scrapie infectivity is considerably more efficient than purified PrP[TSE] at inducing persistent PrP[TSE] production in cultured cells [3]. Small membranous particles such as exosomes (Fig. 6) can be released from multivesicular
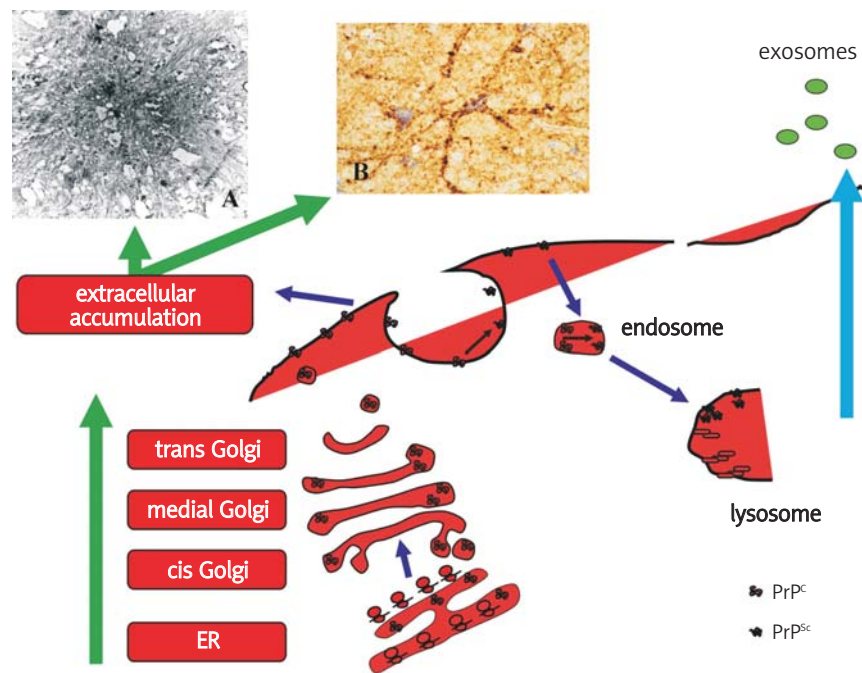


**Fig. 6.** Cellular trafficking of PrP. PrP[c] is synthesized first in the endoplasmic reticulum (ER), matured in the Golgi (cis, medial and trans; red arrow) apparatus, and transported in membrane-bound vesicles to the plasma membrane, where it is anchored by the GPI moiety. Once on the cell surface, PrP[c] can be subjected to endocytosis and either degraded in lysosomes or recycled to the plasma membrane via recycling endosomes. PrP may also be transported through the membrane (yellow arrow) as multivesicular-bodies-derived exosomes. In TSEs, PrP[TSE] accumulates extracellularly (green arrows) as plaques (A) or different forms of deposits (B). A plaque in "A" is a florid plaque of vCJD. Deposits in "B" are perineuronal deposits from Echigo-1 strain of CJD passaged in Syrian hamsters

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown

A

0.5 μm

B

0.5 μm

Fig. 7. (A) Synaptic pattern of PrP^TSE deposition and (B) plaques in kuru affected brain. Arrows in "A" point to focal accumulations of PrP^TSE. Plaques in "B" are from a kuru specimen kindly provided by D. Carleton Gajdusek. Bars, 0.5 μm

bodies and fuse with the membranes of other cells, providing a potential mechanism for the spread of infectivity between cells [3,4,64].

## Deposition of pathologically misfolded protein

Deposits of misfolded PrP$^{TSE}$ that accumulate within the central (CNS) and peripheral nervous system (PNS) and lymphatic tissues [138] correlate with infectivity in most but not all situations [127,154]. Immunohistochemistry (IHC) has become the major diagnostic tool for human prion diseases, by allowing the detection of PrP$^{TSE}$ in fixed tissue sections [33]. More refined approaches, i.e. the histoblot [211] and *paraffin-embedded blot* (PET) techniques, can also be helpful [196]. A major hurdle with IHC is the elimination PrP$^c$ labelling as no available antibodies, including the widely used and commercially available antibodies, can discriminate between PrP$^c$ and misfolded PrP$^{TSE}$ in tissue sections.

Several patterns of PrP$^{TSE}$ expression are revealed by IHC including synaptic (the most difficult to visualize; Fig. 7a), perivacuolar, perineuronal (Fig. 7) and plaque-like [33]. If amyloid is visualized by routine neuropathology (H & E, Congo red, PAS- or Alcian blue staining), it is designated as "plaques" (Fig. 6, 7b); if detected only by IHC and not visible by routine techniques, it is called "plaque-like deposits".

## Neuronal cell death in TSEs

The premature, primary death of nerve cells underlies the clinical symptoms of prion diseases. Unfortunately, despite the great efforts of researchers, the cellular pathways leading to this neuronal loss are not entirely clear. What is more, the question whether there is a direct relation between the deposits of PrP$^{TSE}$ and the loss of neurons still remains conjectural. As in other neurodegenerative diseases, in TSEs apoptosis has become the most popular concept of cell death. However, there is no direct and convincing evidence of apoptosis of nerve cells in most of the neurodegenerative diseases. In addition, the term "apoptosis" is used in a wider sense than it was originally coined and it has become synonymous with non-necrotic cell death or even with programmed cell death. The data on the role of apoptosis in prion diseases are conflicting. Among recently recognized other types of programmed cell death only autophagy has been reported in TSEs but its role in prion diseases pathology is not established.

There is currently no consensus on the classification of different types of programmed cell death. One of the oldest but also still considered most accurate classifications is based on morphology. According to this classification introduced by Schweichel and Merker [197], three types of programmed cell death (PCD) are discriminated [116,189,215,230]:
1) apoptosis,
2) autophagy,
3) cytoplasmic cell death.

Although it must be stressed that this categorization was based on the ultrastructural features of embryonic cells during morphogenesis, such a classification is widely accepted for developing and mature organisms.

1. The term "apoptosis" in its original meaning refers to a morphological phenomenon [111] characterized by chromatin condensation, cell shrinkage, pyknosis, plasma membrane blebbing and fragmentation of the nucleus (karyorrhexis). There is little or no ultrastructural modification of other subcellular organelles. The integrity of plasma membrane is maintained until the late stages of the process [124]. In the end-stage, the cell breaks into small membrane-bound fragments, called apoptotic bodies, which are phagocytosed by macrophages or, in the case of neurons, by microglial cells without inciting any inflammatory response [111]. Apoptosis is regulated by a highly conservative network of molecules consisting of Bcl-2 family and caspases and Apaf-1 [116,189,215,230]. The apoptotic cell death process has a very rapid time course and is complete within a few hours.

2. Autophagic cell death is also one of the programmed cell death mechanisms, sometimes called "type II programmed cell death", in contrast to "type I programmed cell death" (apoptosis). Contrary to apoptosis, autophagic cell death is characterized by abundant autophagic vacuoles in the cytoplasm, mitochondrial dilatation, and enlargement of both the Golgi apparatus and endoplasmatic reticulum, which precedes nuclear destruction. Intermediate filaments and microfilaments are largely preserved [36,37,99].

3. A third type of programmed cell death is called "cytoplasmic cell death" and it was subsequently

divided into two subtypes, 3A and 3B [49]. Type 3A of neuronal death that occurs during development is characterized by swelling of subcellular organelles, formation of large empty spaces within the cytoplasm, and fusion of these spaces to extracellular space and, finally, disintegration of the cellular structures. There are no features of autophagic or heterophagic activity. In the 3B subtype of cell death there are similar vacuoles and empty spaces in the cytoplasm but, in addition, there is a retraction of plasma membrane and karyolysis.

According to a recent review [162], it is now possible to discriminate eleven pathways of cell death occurring in mammals. These types of cell death include: 1) necrosis, 2) apoptosis, 3) anoikis , 4) caspase-independent apoptosis, 5) autophagy, 6) Wallerian degeneration, 7) excitotoxicity, 8) erythropoiesis, 9) platelet cell death, 10) cornification and 11) lens cell death. All of them, except for necrosis, are genetically programmed; however, morphological features of necrosis are occasionally observed during the active cell processes [158]. Some of those subtypes occur merely in one type of cell but others are more common. The first seven types are observed in nerve cells. The latter classification is also based on morphological features because in the majority of cases the molecular mechanism is not known [162]. Other investigators have discriminated more types of cell death, e.g. paraptosis, pyroptosis, oncosis, abortosis, aposklesis and many more [66,168,188,205,228]. Recently, the Nomenclature Committee on Cell Death has been established and according to its suggestions the whimsical names of cell death should be replaced by more descriptive terms [123].

## Prion proteins and the cell death

Mechanistically, neuronal cell death in prion disease has a remarkable feature that distinguishes it from neuronal loss in other neurodegenerative disorders. In the absence of cellular expression of PrP$^c$, neuronal death does not occur [103]. This was initially shown in cell culture systems with neurones derived from PrP-knockout mice [23,78], and later confirmed using an animal model in which PrP$^{TSE}$ was transplanted into PrP-deficient tissue without evidence of neuronal death [15]. Even more

complicated genetically engineered models have demonstrated that when cellular expression of PrP$^c$ is switched off, cell loss does not occur, and disease progress is abated; illness is entirely prevented when neurones cannot produce PrP$^c$, even when non-neuronal tissue contains dense deposits of PrP$^{TSE}$.

Neuronal protection against cell death can also occur in an animal model in which a different form of PrP$^c$ is expressed that is more difficult to convert to PrP$^{TSE}$ because of a species barrier. In transgenic mice co-expressing hamster and mouse PrP$^c$, infection with hamster prion results in production of PrP$^{TSE}$ without any pathology or neuronal death [187]. This suggests that expression of a form of PrP$^c$ resistant to protein conversion protects cells from neurotoxicity. However, if the transgenic mice only express hamster PrP$^c$ in astrocytes, then infection with hamster prions results in prion diseases with associated neuronal death. In this case, the neuronal death is possibly indirect as a result of increased neuronal sensitivity to glutamate toxicity [18].

The potential for PrP$^c$ expression to protect against neuronal cell death runs counter to the finding that expression is necessary for susceptibility to neuronal death in prion disease. The only logical explanation is that its normal cellular function is protective. Loss of that function due to loss of expression of the protein would result in alterations in the expression of proteins with a similar function, resulting in a compensatory effect. However, loss of function of the protein with continued expression, as occurs in prion disease, does not result in an increase in the compensatory mechanisms. It has been postulated that PrP$^c$ is an antioxidant (increased expression of other antioxidants has been noted in PrP-knockout mice) [26,219]. Simple loss of function is clearly not the cause of cell death in prion disease, as PrP-knockout mice do not show altered cell survival *in vivo* [34]; however, neurons in culture are more susceptible to a range of neurotoxic insults when they are derived from PrP-deficient mice [24,26,27,126,219].

## Aggresomes

It is well known that protein aggregates are generally difficult to unfold or to degrade. Misfolded and aggregated proteins are usually handled in the cell through chaperone-mediated refolding or, when this is impossible, they are destroyed by proteasomal degradation. Recent findings suggest that there is

a third way for a cell to deal with misfolded proteins. This pathway involves the sequestration of aggregated proteins into specialized "holding stations", as they are sometimes called, or aggresomes. In this mechanism proteins form small aggregates that are transported along microtubules (MTs) towards a microtubule organizing centre (MTOC) by a process mediated by the minus-end motor protein dynein. At the organizing centre the particles form a spherical structure, usually 1-3 μm in diameter, called an aggresome. Aggresomes are not just static garbage deposits; they recruit various chaperones, ubiquitination enzymes, and proteasome components. They are also supposed to trigger autophagy [74].

A recent report of Kristiansen et al. [122] suggests that neuronal propagation of prions invokes a neurotoxic mechanism involving intracellular formation of PrP$^{TSE}$ aggresomes. The authors showed that only in prion-infected cells did mild proteasome impairment result in formation of large cytosolic, perinuclear structures, containing PrP$^{TSE}$, heat shock protein 70, ubiquitin, proteasome subunits and vimentin. These structures are consistent with the definition of aggresomes. Those authors also claimed to show aggresomes *in vivo* in brains of prion-infected mice, but it is well known that vimentin is present in neurons only in trace quantities while it is robust in glial cells. This means that showing aggresomes *in vivo* needs further studies. A few years earlier Cohen and Taraboulos [50] showed that hampering the activity of cyclophilin isomerases with the fungal immunosuppressant CsA in different cell lines led to accumulation of a PrP population with prion-like properties that was not ubiquitylated and partially resisted proteasomal degradation. These aggregated molecules formed perinuclear aggresomes. Although a growing body of evidence seems to confirm the formation of aggresomes in prion diseases, it must be mentioned that the majority of the studies were performed *in vitro* and PrP$^{TSE}$ in human or animal diseased brains does not intracellular aggregates reminiscent of aggresomes. Similar aggregates are only observed by electron microscopy, but they are extremely rare.

## PrP and Oxidative Stress

Recent work on cell death mechanisms has focused on pathways involving PrP$^{TSE}$. Numerous cell culture lines exposed to neurotoxic PrP fragments have not always given consistent results, although it appears that caspases are involved [96,122,191,201], and that levels of ERK proteins are increased when cells are treated with toxic forms of PrP [129]. Other proteins have also been suggested to play a role, such as p38 [52], JNK, and Bax. However, none of these observations have been independently confirmed. Several of the studies have also suggested that calcium entry is involved [24,172,175,213], but this is a common event of many cell death pathways and the true intracellular pathway has yet to be determined.

It seems likely that some extracellular event must trigger the intracellular pathway that leads to cell death, and many researchers have attempted to identify binding partners on the cell surface that could initiate the intracellular process. Several proteins have been suggested and include the laminin receptor [190], stress inducible protein-1 [231], and PrP$^c$ [20]. Binding of PrP106-126 to PrP$^c$ causes direct inhibition of the antioxidant activity of PrP$^c$ [20]. Treatment of neurons with PrP106-126 causes a marked reduction in the resistance of neurons to oxidative stress [94], and a decrease in the activity of other antioxidant enzymes such as Cu/Zn superoxide dismutase [30,219]. In addition, the peptide causes a decrease in the uptake of Cu by neuronal cells [20], and a decrease of Cu incorporation into Cu/Zn superoxide dismutase [21]. It is unclear how these changes are brought about. However, there is evidence that PrP106-126 can enter cells [160] and might interact with intracellular proteins in microtubules [28], and aggregates of PrP106-126 can cause PrP$^c$ to become trapped in the aggregates [20].

Interestingly, a subset of antibodies to PrP can also cause *in vitro* apoptosis, suggesting that inhibition of protein catabolism or its interaction with other proteins might be sufficient to trigger cell death [29]. Recently, it has been shown that similar antibodies injected *in vivo* can also cause neuronal apoptosis [203]. As antibodies to PrP increase the toxicity of Cu ions to cells, it is possible that interference with the protein's role in Cu metabolism might be central to the ability of PrP106-126 to initiate cell death. However, there is evidence to suggest that the direct effect of PrP106-126 on neurons is insufficient to complete its execution. PrP106126 also has the effect of compromising the neurons' ability to deal with stressful conditions [27], and in this way gives neurons a phenotype like that observed for PrP-deficient neurones [26].

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown

Execution of cell death then comes about as a result of this compromised phenotype and one of a number of different stress events such as the production of superoxide [27].

Several studies have identified markers of oxidative stress in the brains of rodents with prion disease. There are increased levels of oxidised lipids in the brains of scrapie-infected hamsters [86]. Another study [87] has shown increased levels of nitrotyrosine and hemeoxygenase-1 in the brains of scrapie-infected mice. These observations imply that significant free radical damage is being generated in the brains of scrapie-infected mice. Additionally, there is evidence for mitochondrial damage in cells from brains of scrapie-infected hamsters and mice [48,128]. These changes include reduction in the activity of mitochondrial enzymes and structural abnormalities in the mitochondria. Other enzymes known to be associated with resistance to oxidative stress, such as catalase and glutathione-S-transfe-rase, show increased expression [128].

Taken together, these results suggest that oxidative stress is involved in the pathology of prion diseases. It is possible that oxidative damage to the brain in scrapie might be a result of damage to the mitochondria, which can generate superoxide. However, the measured level of oxygen radicals detected with dichlorofluorescein in mitochondrial fractions from the brains of scrapie-infected mice was not greatly increased above that of controls [112]. Reactive oxygen species such as superoxide are generated by microglia and the implication of this is that microglia cause damage in the brain of scrapie-infected mice. *In vitro* studies have de-monstrated that microglia can mediate the cell death caused by PrP$^{TSE}$ or PrP106-126 [27,78,155, 156]. However, microglia activation has also been postulated to be a response to neuronal damage rather than the cause of it. Even if neuronal damage was the sole cause of the microglial response (which is unlikely given the complexity of cell-cell interactions), then the microglial activation is still likely to cause significant production of toxic substances to trigger neuronal apoptosis.

In summary, the neurotoxic mechanism involved in prion disease remains unresolved. However, it is clear that PrP$^c$ plays a major role in the mechanism, making cells susceptible to cell death. Cell death could involve loss or subversion of the normal cellular function of the protein and could make cells susceptible to the toxicity of a variety of agents such as superoxide released by microglia. Therefore a combination of direct and indirect effects caused by PrP$^{TSE}$ remains the most likely explanation of the cell death mechanism.

## Apoptosis and (macro)autophagy

Using TUNEL methodology, apoptotic neurons have been repeatedly identified in both naturally occurring and experimentally induced TSEs [60,69, 70,107-109], and some investigators believe that at least a proportion of "dark neurons" that are shrunken, with homogeneously dark cytoplasm, may represent cells undergoing apoptosis (other workers regard them as fixation artefacts). The data on whether neurons, and perhaps even glia, die of apoptosis in TSE are conflicting, however. While Migheli et al. [165] found no evidence of apoptosis in scrapie-infected mouse brains, Giese et al. [79,80], Lucassen et al. [146], Fraser et al. [70], Williams et al. [220], Kretzschmar et al. [119-121], and Jamieson et al. [100] readily found apoptosis in various scrapie-infected rodent models. The characteristic DNA fragmentation ladder has been seen in both BSE [212] and natural scrapie [62], and *in situ* end-labelling (ISEL) revealed apoptotic cells in human and experimental CJD [63,84,107]. Collectively, these data strongly suggest that neurons in TSEs die because of apoptosis.

It is evident that PrP$^{TSE}$ accumulation in TSE-affected brain largely precedes development of other changes – i.e. spongiform change and astrogliosis, for which PrP serves as a signal for proliferation [17,18,22,25,88,229]. At the ultrastructural level, apparently normal looking neurons secrete PrP$^{TSE}$, which later fibrillizes and becomes neurotoxic [104], and *in vitro* experiments highlight some possible intermediate steps.

As mentioned, several synthetic peptides which form amyloid fibrils, e.g. PrP106126 [19] and PrP118-135 [179], induce apoptosis in a dose-dependent manner. PrP106-126 may exert its pro-apoptotic characteristics via disruption of mitochondrial membranes with subsequent release of cytochrome-c and caspase activation [175]. Next, intracellular Ca$^{2+}$ concentration rises and another family of proteases, calpains, are activated. In contrast, Bounhar et al. [14] found that PrP may serve as an anti-apoptotic factor protecting neurons *in vitro* from Bax-induced apoptosis. Removal

**Fig. 8.** An autophagic vacuole containing a part of the cytoplasm. Phagophore is indicated by arrows; mt, microtubules. A hamster brain infected with the Echigo-1 strain of CJD. Original magn. × 50 000; bar = 100 nm

of four of five octarepeats (codon 51-91 of PrP) or D178N and T183A *PRNP* mutations completely abolished this effect. It is apparent that a functional gain of PrP (as PrP[TSE]) may result in changes of anti-apoptotic into pro-apoptotic properties of PrP, which is vaguely reminiscent of changes of anti-oncogenic into oncogenic functions of p53 protein (also involved in apoptosis).

Moreover, there is no consistent correlation between PrP[TSE], pathology and neuronal death. Following transmission of BSE from cattle to mice, Lasmezas et al. [127] found two lines of BSE-infected mice, one with PrP[TSE] and typical TSE pathology (vacuolation and astrogliosis), and a second without PrP[TSE] but with prominent apoptosis of neurons. These observations suggest that PrP[TSE] is not directly responsible for apoptosis.

However, in another experiment, where neuronal PrP[c] was depleted in NFH-Cre/M*lox*P transgenic mice, after early spongiform change had developed following scrapie neuroinvasion, Collinge's group [151] found a reversal of spongiform change, while PrP[TSE], released probably from glial cells, steadily accumulated. These PrP[c]-depleted Tg mice also became disease-free; thus, the lack of neuronal PrP[TSE] and/or PrP[c] protected them from the final prion-related neuronal degeneration. In contrast, in transgenic mice in which PrP transgene was exclusively expressed in astrocytes under the control of GFAP promoter, spongiform change and neuronal decay were observed following scrapie infection [103].

In an independent experiment, the highest densities of apoptotic cells were observed in those neuroanatomical areas in which spongiform change is minimal or absent – viz. retina and cerebellum [120]. Thus, apoptosis and the presence of PrP[TSE] may not only be uncoupled under specific experimental conditions but may be directly (not mediated through PrP[TSE]) linked to scrapie infectivity.

Neurons may only degenerate and eventually die through a limited number of pathologic pathways. Cellular necrosis is caused by a sudden brain insult,

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown
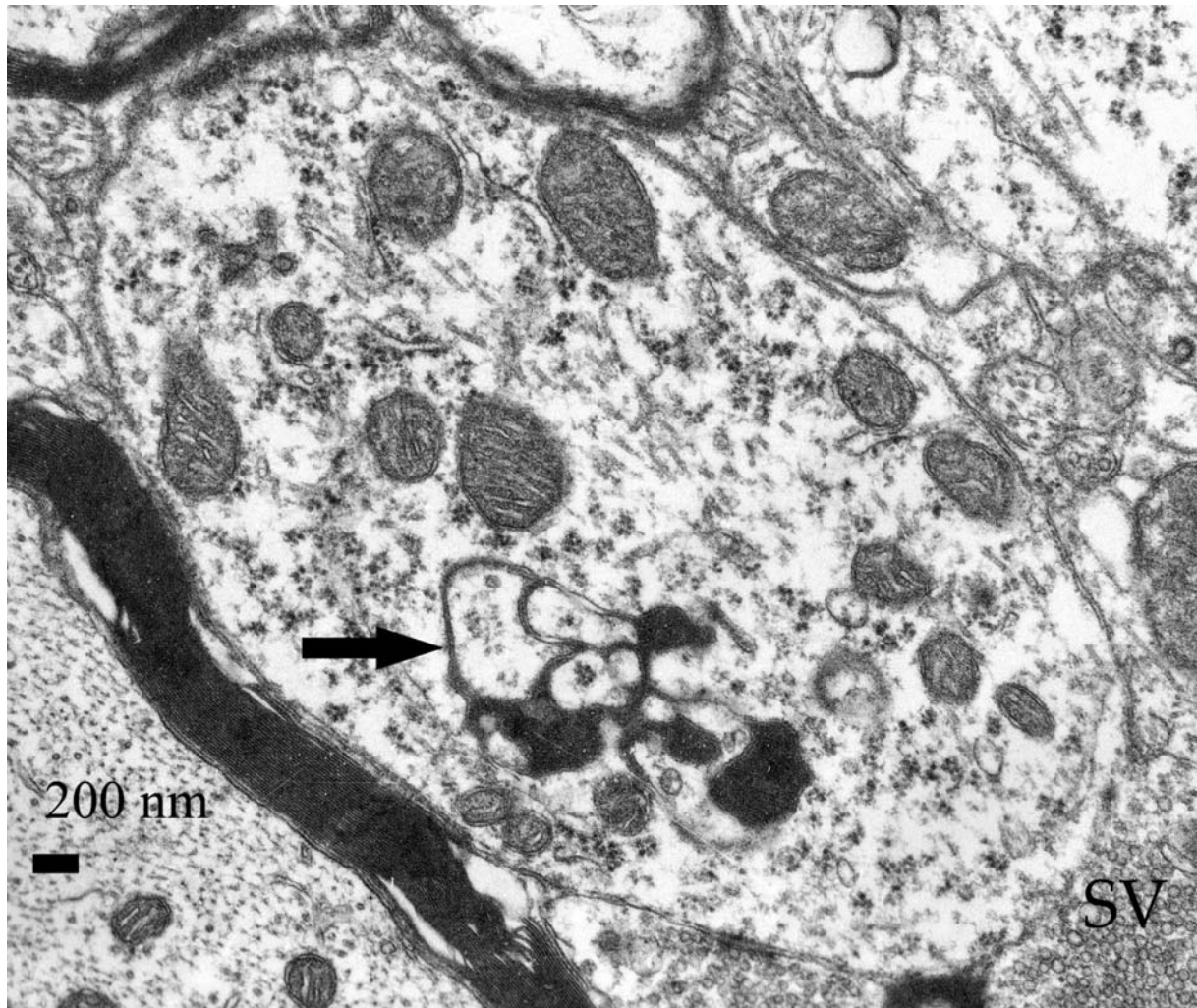


**Fig. 9.** Neuronal autophagic vacuoles in an early stage of development. Note that sequestrated cytoplasm is of high electron density (arrow). SV – synaptic vesicles. A hamster brain infected with the 263K strain of scrapie. Original magn. × 12 000; bar = 200 nm

leading to destruction of the entire cell, the remnants of which attract inflammatory cells. Apoptosis is a programmed molecular process following a "suicidal" stimulus, which leads to a hierarchical gene response. Apoptotic cells, in contrast to necrotic cells, attract no inflammatory response. As the relative lack of classical immunological response in TSE-affected brain is paradigmatic for the whole group of these diseases [31], neurons, by definition, should die by apoptosis and not necrosis.

A determinant for apoptotic cell detection is the time over which neurons die. Even when the number of neurons in dorsal lateral geniculate nucleus (dLGN) following intraocular inoculation drops from over 22 000 to less than 2000 [101], the number of apoptotic nuclei detected by TUNEL method is low [70]. Because apoptotic cells are most readily detected in highly structured neuronal systems such as the retina and hippocampus, what is observable in less structured regions may represent only a minute proportion of all apoptotic events occurring in TSE-affected brains.

As an evolutionarily ancient cellular response to intra- and extracellular noxious stimuli, autophagy may precede or co-exist with apoptosis, and the process may be induced by apoptotic stimuli [38,227]. Furthermore, the level of autophagy may define the sensitivity of a given neuronal population to apoptotic
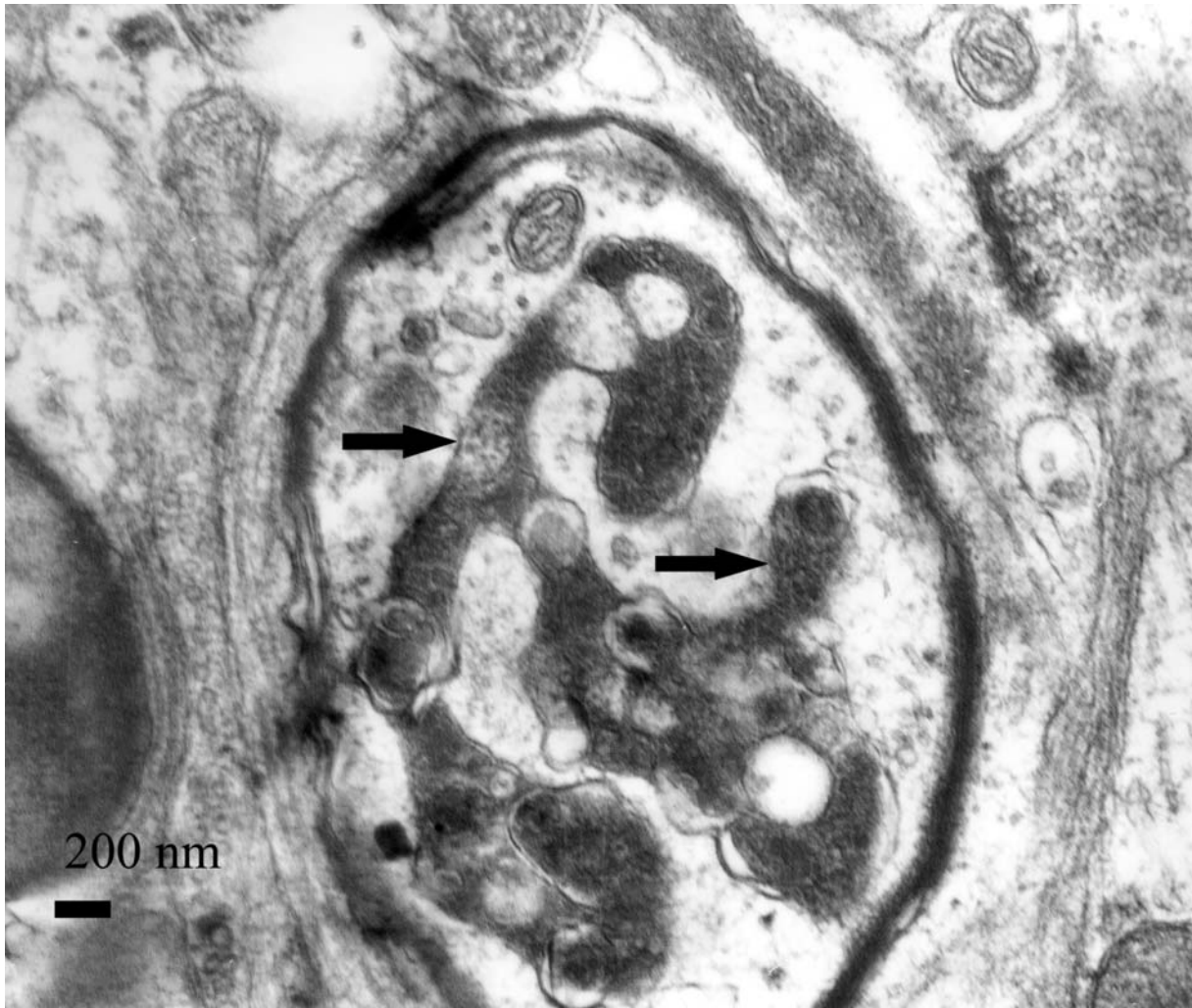
**Fig. 10.** Branching autophagic vacuole within a myelinated fibre. Note that sequestrated cytoplasm is of high electron density. A hamster brain infected with the 263K strain of scrapie. Original magn. × 30 000; bar = 200 nm

stimuli, which may underlie the phenomenon of "selective neuronal vulnerability". Thus, autophagy and apoptosis are often interwoven [40].

Cellular autophagy is a physiological degradation process employed, like apoptosis, in embryonic growth and development, cellular remodelling and the biogenesis of some subcellular organelles – viz. multilamellar bodies [65,91,192]. Autophagosomes coalesce with lysosomes to form degraded autophagic vacuoles, and as in apoptosis, only excessive or misdirected autophagy causes a pathological process. Autophagy is highly enhanced in other brain amyloidoses or conformational disorders [83], Alzheimer's disease [206], Parkinson's disease [2],

and Huntington's disease, in which the signal for autophagy is huntingtin [110]. Here, we extend these observations using different models of scrapie and CJD.

## Neuronal autophagy in TSEs

Data on autophagy in TSEs are very limited, consisting of a few electron-microscopic papers, including reports from our own laboratories [132,136,143-145,199]. The pioneering work was published in Acta Neuropathologica by Boellaard et al. [9]. Our initial experimental approach using the hamster-adapted 263K or 22C-H strains of scrapie

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown
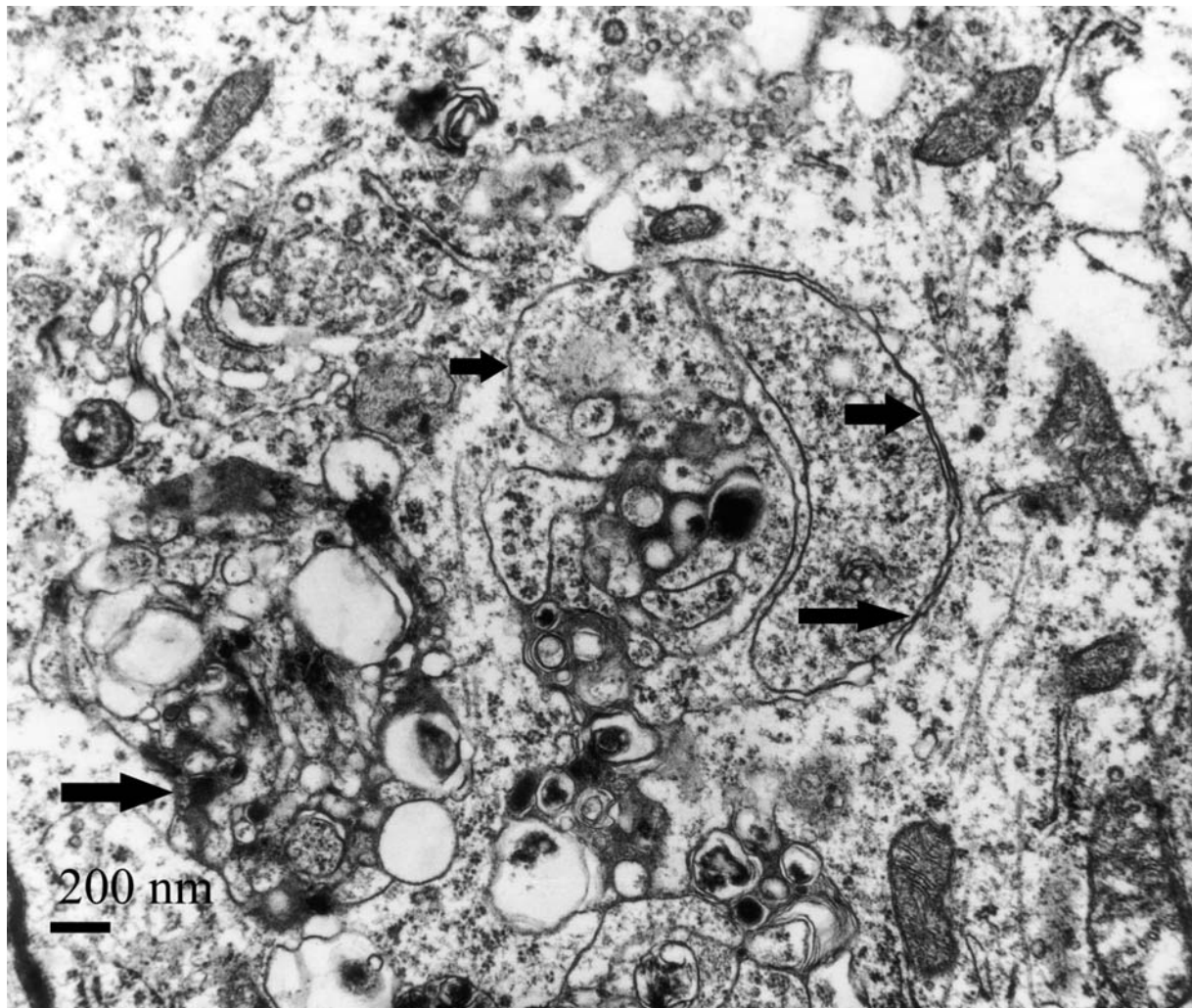
**Fig. 11.** Fully developed autophagic vacuole. Phagophores are pointed to by arrows. Note that part of the sequestrated cytoplasm is of high electron density. A hamster brain infected with the 263K strain of scrapie. Original magn. × 12 000; bar = 200 nm

[132,136,145] was subsequently extended by studies of human brain biopsies from patients with sporadic CJD, variant CJD, and FFI [144]. Experimentally infected animal models are widely used because of their relatively short incubation periods that, for mice, range from 16 to 18 weeks, and for hamsters from 9 to 10 weeks for the 263K strain and 24-26 weeks for the 22C-H strain.

## Formation of autophagic vacuoles in TSEs-affected brains

Autophagic vacuoles are areas of the cytoplasm sequestered within double or multiple membranes (phagophores) of unknown origin; one possible source is the endoplasmic reticulum (Fig. 8). They contain ribosomes, small secondary vacuoles, and occasional mitochondria. Some vacuoles present a homogeneously dense appearance.

We observed neuronal autophagic vacuoles in different stages of formation in the same specimens and our interpretation of the 'maturity' of their formation may or may not equate to actual developmental stages. Initially, a part of the neuronal cytoplasm was sequestered within double or multiple membranes (phagophores) and often exhibited increased electron-density (Figs. 9-11). The intra-cytoplasmic membranes multiplied in a labyrinth-like
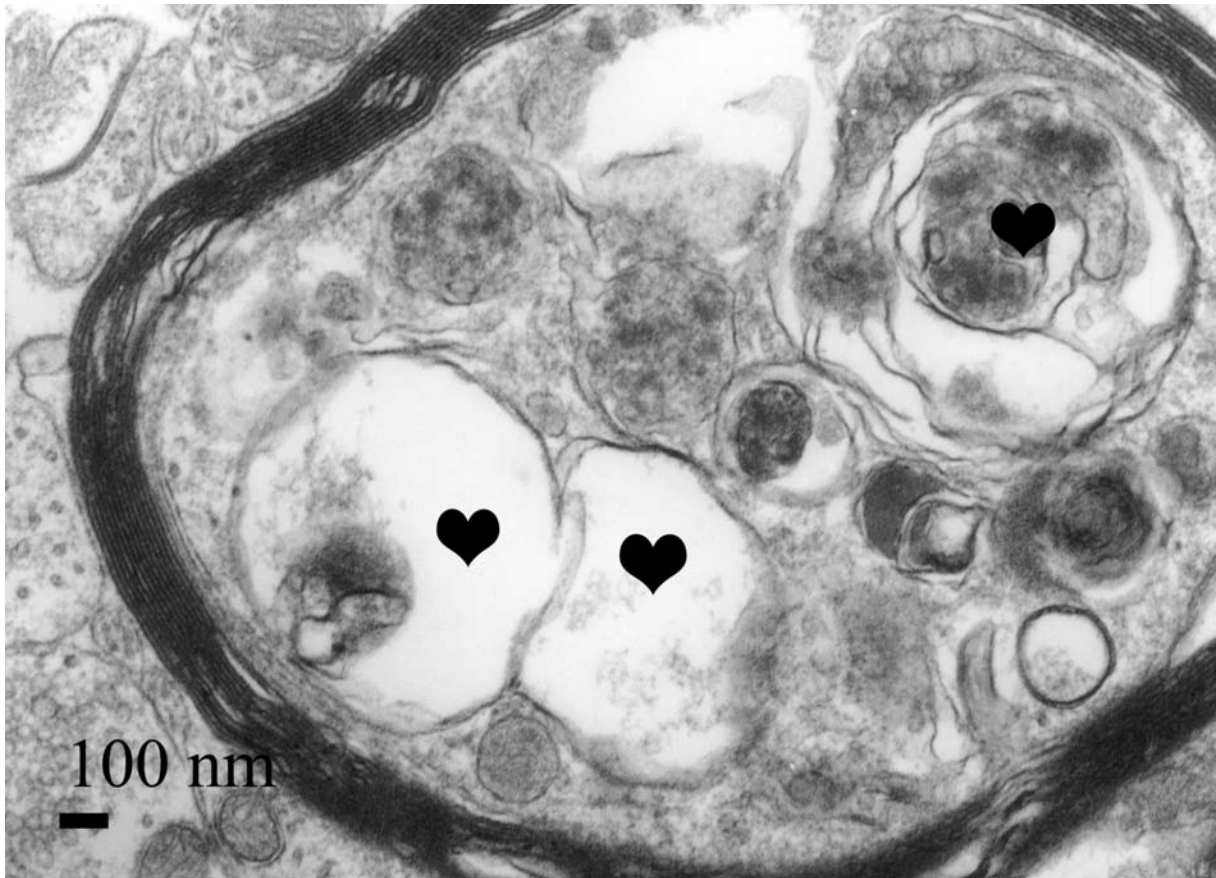
**Fig. 12.** Section through a myelinated fibre showing formation of autophagic vacuoles (hearts). A hamster brain infected with the 263K strain of scrapie. Original magn. × 8300; bar = 100 nm

manner (Fig. 11). The autophagic vacuoles then expanded and eventually a vast area of the cytoplasm was transformed into a merging mass of autophagic vacuoles. Occasionally, a single large autophagic vacuole was visible. Autophagic vacuoles developed not only in neuronal perikarya but also in neuronal processes, eventually replacing the whole cross-section of affected neurites (Fig. 9, 12). In a few specimens we found round electron-dense structures that we identified as aggresomes. In general, there was little qualitative difference between these two models, although hamsters inoculated with the 263K strain showed a more robust pathology.

## Conclusions and hypotheses

One of the major problems of TSEs pathogenesis is the cause of neuronal degeneration with eventual neuronal loss [69,136]. Whether the prion is or is not the pathogen, it is widely accepted that the basic underlying pathological event is the conversion of a normal isoform of prion protein (PrP$^c$) into its pathological misfolded isoform (PrP$^{TSE}$) [15,51,178, 183], involving an $\alpha$-helical to $\beta$-pleated sheath (or $\beta$-helical) transformation. Using a highly sophisticated mathematical model, Stumpf and Krakauer [210] tried to reason whether PrP$^{TSE}$ causes neurons to die because of neurotoxic effect of PrP$^{TSE}$ (gain of function), or loss of function of PrP$^c$. They assumed that if cells die of apoptosis because of neurotoxic gain of function of PrP$^{TSE}$, the cells should die rapidly, and the amount of PrP$^{TSE}$ should be low. Indeed, in both CJD and FFI, there are more apoptotic cells and a lower amount of PrP$^{TSE}$ [107] than in GSS, where the amount of amyloid is vast and the number of apoptotic cells is low [84].

As already mentioned, three types of programmed cell death (PCD) are known: apoptosis, autophagy and swelling of intracellular organelles. Apoptosis has

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown
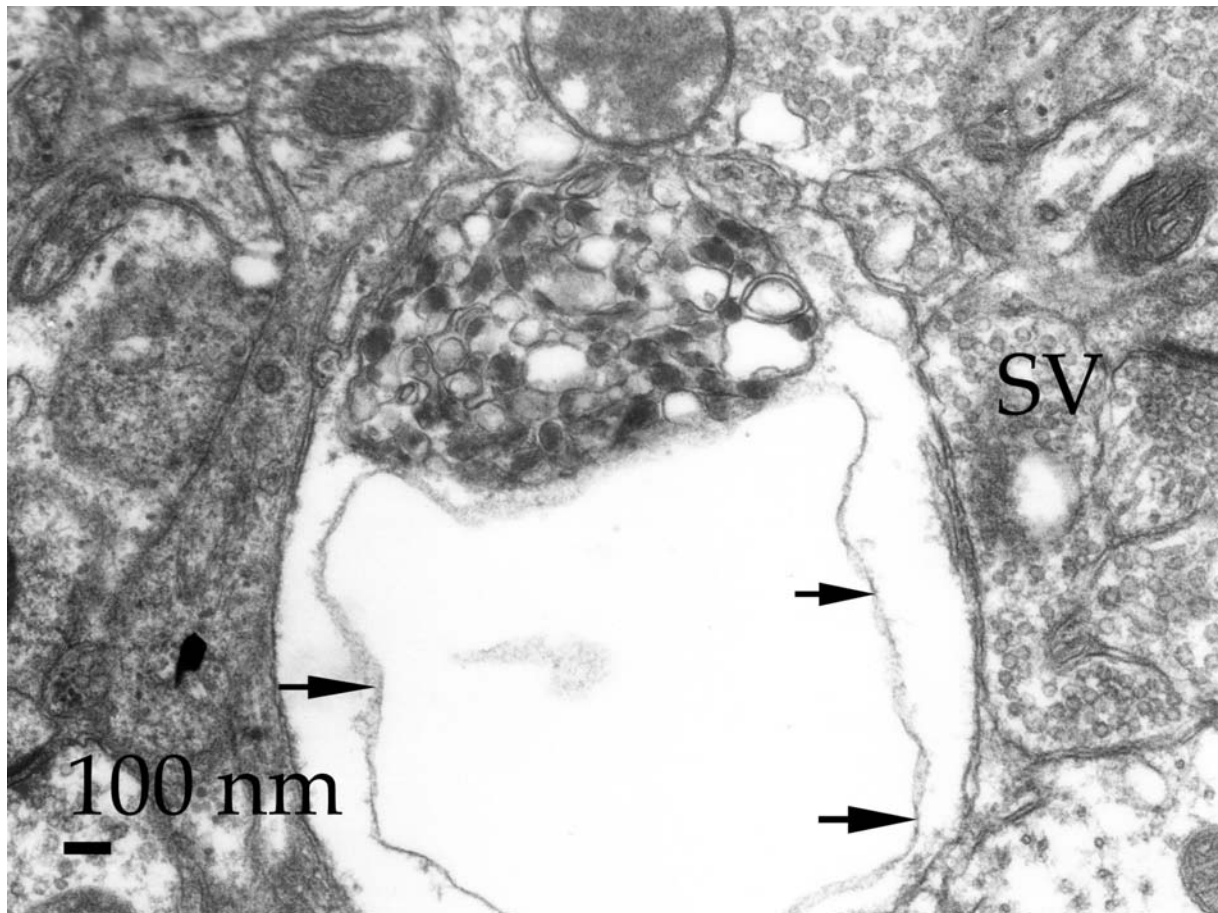


**Fig. 13.** "Spongiform" vacuoles within a dendrite (because a synaptic terminal with vesicles (SV) is attached to this vacuole) containing a structure at the top suggestive of undergoing autophagy. Note that sequestrated cytoplasm is of high electron density. Curled membrane fragments are indicated by arrows. A hamster brain infected with the 263K strain of scrapie. Original magn. × 8300; bar = 100 nm

been discussed already, and in this part we will concentrate on autophagy in regard to TSEs.

Whereas apoptosis in TSE is relatively well understood, autophagy is not. As already mentioned, autophagic cell death or type II PCS is a process used by a cell to remove the bulk of organelles, e.g. during growth and development, but which becomes pathological if too robust or wrongly placed [39]. According to Bursch et al. [39], the appearance of "autophagic vacuoles in dying cells by electron microscopy is taken as condition sine qua non (i.e. an absolute prerequisite) to denote cell death as autophagic/type II PCD". To this end, the mere presence of autophagic vacuoles is relatively well demonstrated in TSE.

There are several uncertainties in our thinking on neuronal autophagy in TSEs. First, autophagy is regarded as a short-term response to nutrient limitations [61], which is, by definition, not the case in slow transmissible diseases like TSEs. However, it seems that autophagy is activated to prevent apoptosis; when autophagy is blocked, apoptosis ensues. On the other hand, when apoptosis is blocked as in Bax/Bak-deficient mice, autophagy is activated as a cell-survival mechanism [148]. However, when all subcellular organelles are self-eaten, the cell eventually dies. An analogous situation was observed when inhibition of autophagosomes and lysosomes was accomplished via targeting of *LAMP2* by RNA interference [82]. A dual role for autophagy was envisaged – i.e. protective role against apoptosis and detrimental role in cell death – and both of these are mediated by the same set of *ATG* genes [61]. Thus, the
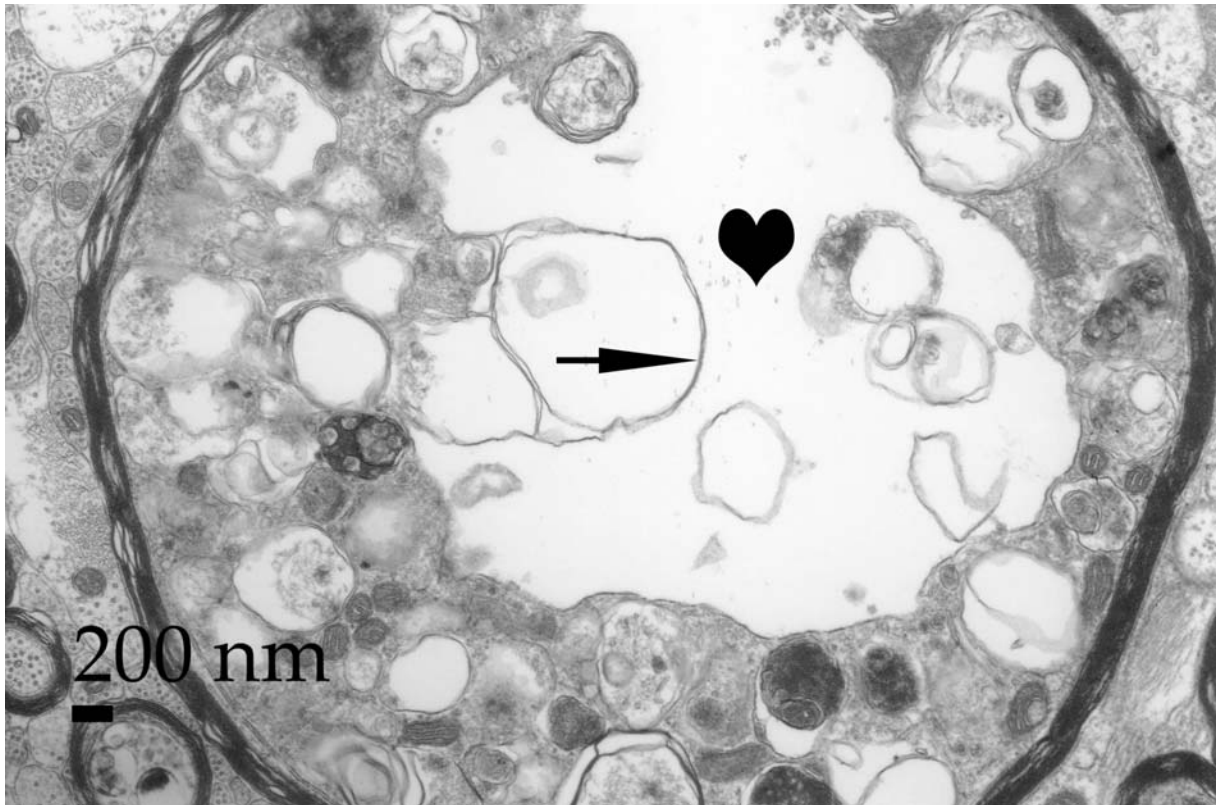
**Fig. 14.** Myelinated process filled with numerous autophagic vacuoles. Centre of the process is already cleared (heart) to form a spongiform vacuole; the curled membrane fragments (an arrow) are visible. A hamster brain infected with the 263K strain of scrapie. Original magn. × 30 000; bar = 200 nm

abundant presence of autophagy in TSEs suggests that neurons either try to escape apoptosis or die by autophagy via one of the programmed-cell death pathways.

A second uncertainty pertains to the relationship between autophagy and the abnormal processing of PrP. The significance of autophagy for controlling the outcome of misfolded PrP$^{TSE}$ is unclear. PrP$^c$ is entirely translocated into the lumen of the endoplasmic reticulum where the N-terminal signal peptide is cleaved away and the C-terminal GPI is added (Figs. 3-4) [92]. Then, PrP$^c$ is transported on the cell surface. The misfolded PrP$^{TSE}$ is localized to the cell surface and the endosomal-lysosomal compartment (Fig. 6); and the conversion of PrP$^c$ into PrP$^{TSE}$ presumably takes place somewhere within one or more of these subcellular compartments [13,41,42]. However, misfolded PrP$^{TSE}$ is directed to the aggresomes where, according to the hypothesis put forward by Cohen and Taraboulos [50], it may form a "seed" for seeded-nucleation process of forming

more PrP$^{TSE}$. Then, aggresomes are engulfed by autophagic vacuoles that, in turn, fuse with lysosomes containing PrP$^{TSE}$, and the process of nucleation may either be initiated or it is perpetuated. It was found that aggresomes may indeed induce the formation of autophagosomes [117].

A third uncertainty is how autophagy contributes to overall pathology underlying TSEs. The hallmark of TSEs is the vacuole which is intracellular "empty" space surrounded by a single or a double membrane. The histogenesis of vacuoles is not well understood and most ultrastructural studies suffer from the inability to ascertain the subcellular organelles from which vacuoles originate; dilated endoplasmic reticulum or mitochondria have been suggested [102]. Our own unpublished work suggests that vacuoles are formed relatively abruptly with no detectable transitional stages [Gibson and Liberski, personal communication]. It is tempting to speculate that vacuolation in TSEs is somehow related to type III PCD characterized by the presence of large, membrane-

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown
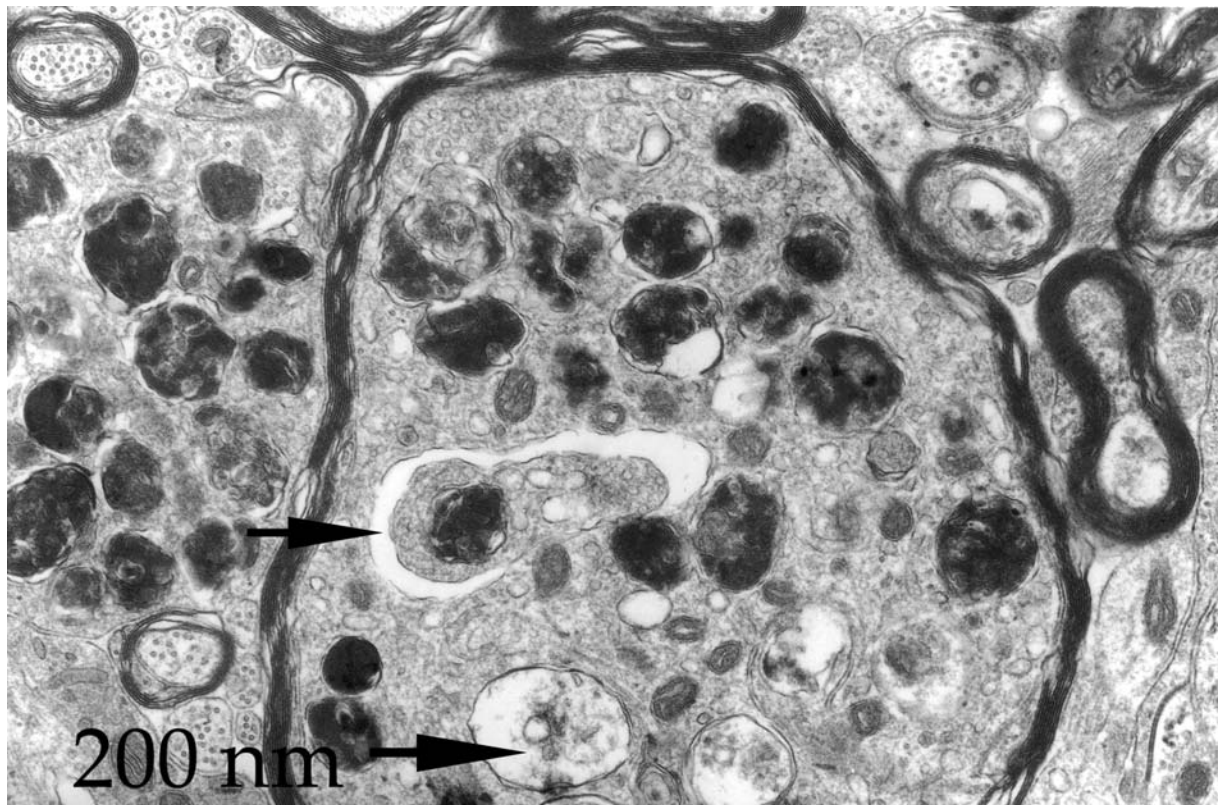


**Fig. 15.** Two dystrophic neurites filled with numerous autophagic vacuoles (arrows) and lysosomal electron-dense bodies. A hamster brain infected with the 263K strain of scrapie. Original magn. × 30 000; bar = 200 nm

bound intracellular empty spaces without the participation of lysosomes. The other option, that tissue destruction by autophagy results in vacuolation (Figs. 13, 14), would imply that autophagic vacuoles should be detected prior to spongiform vacuoles. Indeed, in the terminal stages of sheep scrapie [M. Jeffrey – personal communication] and in human TSEs (CJD, GSS and FFI) [144], little or no autophagy is seen but robust vacuolation is typical if not pathognomic. In contrast, when earlier stages of TSE have been monitored in experimental rodent models, both autophagy and vacuolation have been reported [105,142,145]. Jeffrey et al. [105] have suggested that their presence reflects the robust accumulation of misfolded PrP$^{TSE}$, overloading of the neuronal catabolic machinery, and, eventually, bulk removal of damaged neurons via autophagy.

If this scenario is correct, the pathology of TSEs is akin to the reactivation of certain embryonic processes in which bulk removal of cells are present; i.e. remodelling of insect larvae [173]. Nearly 20 years

ago, Elizabeth Beck [8] suggested that robust vacuolation like that characteristic of TSEs is present when rats are inoculated with a suspension of cells from normal immature cerebellum. Neurons of inoculated rats not only demonstrated intracytoplasmic vacuoles but, at the ultrastructural level, showed abundant coated pits and vesicles – phenomena of widespread appearance in TSEs.

The other form of neuronal degeneration is neuroaxonal dystrophy [77,131,133,134]. The ultrastructural correlate of NAD is the dystrophic neurite – an axon or dendrite filled with electron-dense inclusions, many of which were recently recognized as small autophagic vacuoles (Fig. 15) [174]. Both immature autophagic vacuoles immunogold-labelled with abs against Cat-D and mature vacuoles containing catepsin were observed within those neurites [174]. As dystrophic neurites are abundant in TSEs [131,141], it is plausible that (macro)autophagy plays a role in neuronal degeneration in TSEs. However, taking into account the evidence that autophagy may prevent

apoptosis, it is also possible that abundant presence of autophagic vacuoles within dystrophic neurites actually reflects neurons struggling to survive in the noxious environment of misfolded PrP^TSE.

In summary, autophagy certainly does occur in TSE, but its pathogenetic role as a cause of cell death is uncertain. In particular, more research will be necessary to determine the connection, if any, between programmed cell death and the formation of spongiform change.

### Acknowledgements

### References

1. Alper T, Cramp WA, Haig DA, Clarke MC. Does the agent of scrapie replicate without nucleic acid? Nature 1967; 214: 764-766.

2. Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 1997; 12: 25-31.

3. Baron GS, Magalhaes AC, Prado MA, Caughey B. Mouse-adapted scrapie infection of SN56 cells: greater efficiency with microsome-associated versus purified PrP-res. J Virol 2006; 80: 2106-2117.

4. Baron GS, Wehrly K, Dorward DW, Chesebro B, Caughey B. Conversion of raft associated prion protein to the protease-resistant state requires insertion of PrP-res (PrP(Sc)) into contiguous membranes. EMBO J 2002; 21: 1031-1040.

5. Bartz JC, Kincaid AE, Bessen RA. Retrograde transport of transmissible mink encephalopathy within descending motor tracts. J Virol 2002; 76: 5759-5768.

6. Bartz JC, Kincaid AE, Bessen RA. Rapid prion neuroinvasion following tongue infection. J Virol 2003; 77: 583-591.

7. Basler K, Oesch B, Scott M, Westaway D, Wälchli M, Groth DF, McKinley MP, Prusiner SB, Weissmann C. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell 1986; 46: 417-428.

8. Beck E. Lesions akin to transmissible spongiform encephalo-pathy in the brains of rats inoculated with immature cerebellum. Acta Neuropathol 1988; 76: 295-305.

9. Boellaard JW, Kao M, Schlote W, Diringer H. Neuronal autophagy in experimental scrapie. Acta Neuropathol 1991; 82: 225-228.

10. Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. Science 1982; 218: 1309-1311.

11. Borchelt DR, Koliatsos VE, Guarnieri M, Pardo CA, Sisodia SS, Price DL. Rapid anterograde axonal transport of the cellular prion glycoprotein in the peripheral and central nervous systems. J Biol Chem 1994; 269: 14711-14714.

12. Borchelt DR, Scott M, Taraboulos A, Stahl N, Prusiner SB. Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. J Cell Biol 1990; 110: 743-752.

13. Borchelt DR, Taraboulos A, Prusiner SB. Evidence for synthesis of scrapie prion protein in the endocytic pathway. J Biol Chem 1992; 267: 16188-16199.

14. Bounhar Y. Prion protein protects human neurons against Bax-mediated apoptosis. J Biol Chem 2001; 276: 39145-39149.

15. Brandner S, Isenmann S, Raeber A, Fischer M, Sailer A, Kobayashi Y, Marino S, Weissmann C, Aguzzi A. Normal host prion protein necessary for scrapie-induced neurotoxicity. Nature 1996; 379: 339-343.

16. Bratosiewicz-Wasik J, Wasik T, Liberski PP. Molecular approaches to mechanisms of prion diseases. Folia Neuropathol 2004; 42: 33-46.

17. Brown DR. Prion protein-overexpressing cells show altered response to a neurotoxic prion protein peptide. J Neurosci Res 1998; 54: 331-340.

18. Brown DR. Prion protein peptide neurotoxicity can be mediated by astrocytes. J Neurochem 1999; 73: 1105-1113.

19. Brown DR. Prion protein peptides: optimal toxicity and peptide blockade of toxicity. Mol Cell Neurosci 2000; 15: 66-78.

20. Brown DR. PrPSc-like prion protein peptide inhibits the function of cellular prion protein. Biochem J 2000; 352: 511-518.

21. Brown DR, Besinger A. Prion protein expression and superoxide dismutase activity. Biochem J 1998; 334: 423-429.

22. Brown DR, Besinger A, Herms JW, Kretzschmar HA. Microglial expression of the prion protein. Neuroreport 1998; 9: 1425-1429.

23. Brown DR, Herms J, Kretzschmar HA. Mouse cortical cells lacking cellular PrP survive in culture with a neurotoxic PrP fragment. Neuroreport 1994; 5: 2057-2060.

24. Brown DR, Herms JW, Schmidt B, Kretzschmar HA. PrP and beta-amyloid fragments activate different neurotoxic mechanisms in cultured mouse cells. Eur J Neurosci 1997; 9: 1162-1169.

25. Brown DR, Mohn CM. Astrocytic glutamate uptake and prion protein expression. Glia 1999; 25: 282-292.

26. Brown DR, Nicholas RS, Canevari L. Lack of prion protein expression results in a neuronal phenotype sensitive to stress. J Neurosci Res 2002; 67: 211-224.

27. Brown DR, Schmidt B, Kretzschmar HA. Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. Nature 1996; 380: 345-347.

28. Brown DR, Schmidt B, Kretzschmar HA. A prion protein fragment interacts with PrP-deficient cells. J Neurosci Res 1998; 52: 260-267.

29. Brown DR, Schmidt B, Kretzschmar HA. Effects of copper on survival of prion protein knockout neurons and glia. J Neurochem 1998; 70: 1686-1693.

30. Brown DR, Schultz-Schaeffer WJ, Schmidt B, Kretzschmar HA. Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. Exp Neurol 1997; 146: 104-112.

31. Brown P. The phantasmagoric immunology of transmissible spongiform encephalopathy. In: Waksman BH (ed.). Immunologic Mechanisms in Neurologic and Psychiatric Disease. Raven Press, Ltd, New York, 1990; pp. 305-313.

32. Brown P, Cervenáková L. A prion lexicon (out of control). Lancet 2005; 365: 122.

Paweł P. Liberski, David R. Brown, Beata Sikorska, Byron Caughey, Paul Brown

33. Budka H, Head MW, Ironside JW, Gambetti P, Parchi P, Zeidler M, Tagliavini F. Prion Disorders: CJD: Sporadic Creutzfeldt-Jakob disease. In: Dickson D (ed.). Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders. ISN Neuropath Press, Basel, 2003: pp. 287-297.

34. Bueler H, Fischer M, Lang Y, Bluethmann H, Lipp HP, DeArmond SJ, Prusiner SB, Aguet M, Weissmann C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 1992; 356: 577-582.

35. Burger D, Hartsough GR. A scrapie-like disease of mink. In: Report of a Scrapie Seminar. United States Department of Agriculture, paper 27, 1964; pp. 225-227.

36. Bursch W. The autophagosomal-lysosomal compartment in programmed cell death. Cell Death Differ 2001; 8: 569-581.

37. Bursch W. Multiple cell death programs: Charon's lifts to Hades. FEMS Yeast Res 2004; 5: 101-110.

38. Bursch W, Ellinger A. Autophagy – a basic mechanism and a potential role for neurodegeneration. Folia Neuropathol 2005; 43: 297-310.

39. Bursch W, Ellinger A, Gerner C, Schulte-Hermann R. Autophago-cytosis and programmed cell death. In: Klionsky D (ed.). Autophagy. Landes Bioscience, Georgetown, Texas, USA, 2004; pp. 290-306.

40. Bursch W, Hochegger K, Torok L, Marian B, Ellinger A, Hermann RS. Autophagic and apoptotic types of programmed cell death exhibit different fates of cytoskeletal filaments. J Cell Sci 2000; 113: 1189-1198.

41. Caughey B, Raymond GJ. The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. J Biol Chem 1991; 266: 18217-18223.

42. Caughey B, Raymond GJ, Ernst D, Race RE. N-terminal truncation of the scrapie-associated form of PrP by lysosomal protease(s): implications regarding the site of conversion of PrP to the protease-resistant state. J Virol 1991; 65: 6597-6603.

43. Chen SG, Teplow DB, Parchi P, Teller JK, Gambetti P, Autilio-Gambetti L. Truncated forms of the human prion protein in normal brain and in prion diseases. J Biol Chem 1995; 270: 19173-19180.

44. Chesebro B. Introduction to the transmissible spongiform encephalopathies or prion disease. Brit Med Bull 2003; 63: 1-20.

45. Chesebro B, Race R, Wehrly K, Nishio J, Bloom M, Lechner D, Bergstrom S, Robbins K, Mayer L, Keith JM, et al. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature 1985; 315: 331-333.

46. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, Masliah E, Oldstone M. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science 2005; 308: 1435-1439.

47. Cho HJ. Requirement of a protein component for scrapie infectivity. Intervirology 1980; 14: 213-216.

48. Choi SI, Ju WK, Choi EK, Kim J, Lea HZ, Carp RI, Wisniewski HM, Kim YS. Mitochondrial dysfunction induced by oxidative stress in the brains of hamsters infected with the 263 K scrapie agent. Acta Neuropathol 1998; 96: 279-286.

49. Clarke PG. Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol (Berl) 1990; 181: 195-213.

50. Cohen E, Taraboulos A. Scrapie-like prion protein accumulates in aggresomes of cyclosporin A-treated cells. EMBO J 2003; 22: 404-417.

51. Collinge J. Prion diseases of humans and animals: their causes and molecular basis. Annu Rev Neurosci 2001; 24: 519-550.

52. Corsaro A, Thellung S, Villa V, Principe DR, Paludi D, Arena S, Millo E, Schettini D, Damonte G, Aceto A, Schettini G, Florio T. Prion protein fragment 106-126 induces a p38 MAP kinase-dependent apoptosis in SH-SY5Y neuroblastoma cells independently from the amyloid fibril formation. Ann N Y Acad Sci 2003; 1010: 610-622.

53. Cuille J, Chelle P-L. Pathologie animale – la maladie dite tremblante du mouton est-elle inoculable? Comptes rendus des Seances de l'Academie des Sciences (Paris) 1936; 203: 1552-1554.

54. Cuille J, Chelle P-L. La „tremblante" du mouton est bien inoculable. Comptes rendus des Seances de l'Academie des Sciences (Paris) 1938; 206: 78-79.

55. Cunningham AA, Wells GAH, Scott AC, Kirkwood JK, Barnett JEF. Transmissible spongiform encephalopathy in greater kudu (Tragelaphus strepsiceros). Vet Rec 1993; 132: 68.

56. Deleault NR, Lucassen RW, Supattapone S. RNA molecules stimulate prion protein conversion. Nature 2003; 425: 717-720.

57. Dickinson AG. Scrapie in sheep and goats. In: Kimberlin RH (ed.). Slow Virus Diseases of Animals and Man. North-Holland Publ Comp, Amsterdam, 1976; pp. 209-241.

58. Diringer H. Hidden amyloidoses. Exp Clin Immunogenet 1992; 9: 212-229.

59. Diringer H, Gelderblom H, Hilmert H, Ozel M, Edelbluth C, Kimberlin RH. Scrapie infectivity, fibrils and low molecular weight protein. Nature 1983; 306: 476-478.

60. Dorandeu A, Wingertsmann L, Chretien F, Delisle M-B, Vital C, Parchi P, Montagna P, Lugaresi E, Ironside JW, Budka H, Gambetti P, Gray F. Neuronal apoptosis in fatal familial insomnia. Brain Pathol 1998; 8: 531-537.

61. Eskelinen EL. Doctor Jekyll and Mister Hyde: autophagy can promote both cell survival and cell death. Cell Death Differ 2005; 12 (Suppl 2): 1468-1472.

62. Fairbairn DW, Carnahan KG, Thwaits RN, Grigsby RV, Holyoak GR, O'Neill KL. Detection of apoptosis induced DNA cleavage in scrapie-infected sheep brain. FEMS Microbiol Lett 1994; 115: 341-346.

63. Ferrer I. Nuclear DNA fragmentation in Creutzfeldt-Jakob disease: does a mere positive in situ nuclear end-labeling indicate apoptosis? Acta Neuropathol (Berl) 1994; 97: 5-12.

64. Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, Laude H, Raposo G. Cells release prions in association with exosomes. Proc Natl Acad Sci USA 2004; 101: 9683-9688.

65. Filonova LH, Bozhkov PV, Brukhin VB, Daniel G, Zhivotovsky B, von Arnold S. Two waves of programmed cell death occur during formation and development of somatic embryos in the gymnosperm, Norway spruce. J Cell Sci 2000; 113: 4399-4411.

66. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun 2005; 73: 1907-1916.

67. Flechsig E, Weissmann C. The role of PrP in health and disease. Curr Mol Med 2004; 4: 337-353.

68. Fleetwood AJ, Furley CW. Spongiform encephalopathy in an eland. Vet Rec 1990; 126: 408-409.

69. Fraser JR. What is the basis of transmissible spongiform encephalopathy induced neurodegeneration and it be repaired? Neuropathol Appl Neurobiol 2002; 28: 1-11.

70. Fraser JR, Halliday WG, Brown D, P Belichenko P, Jeffrey M. Mechanisms of scrapie-induced neuronal cell death. In: Court L, Dodet B (eds.). Transmissible subacute spongiform encephalopathies: prion disease. IIIrd International Symposium on transmissible subacute spongiform encephalopathies: prion disease, Val-de-Grace Paris, France. Elsevier, Amsterdam-Oxford-Paris, 1996; pp. 107-112.

71. Gabriel JM, Oesch B, Kretzschmar H, Scott M, Prusiner SB. Molecular cloning of a candidate chicken prion protein. Proc Natl Acad Sci USA 1992; 89: 9097-9101.

72. Gajdusek DC. Unconventional viruses and the origin and disappearance of kuru. Science 1977; 197: 943-960.

73. Gajdusek DC, Gibbs CJ, Alpers M. Experimental transmission of a kuru-like syndrome to chimpanzees. Nature 1966; 209: 794-796.

74. Garcia-Mata R, Gao YS, Sztul E. Hassles with taking out the garbage: aggravating aggresomes. Traffic 2002; 3: 388-396.

75. Gibbons RA, Hunter GD. Nature of the scrapie agent. Nature 1967; 215: 1041-1043.

76. Gibbs CJ Jr, Gajdusek DC, Asher DM, Alpers MP, Beck E, Daniel PM, Matthews WB. Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to chimpanzee. Science 1968; 161: 388-389.

77. Gibson PH, Liberski PP. An electron and light microscopic study of the numbers of dystrophic neurites and vacuoles in the hippocampus of mice infected intracerebrally with scrapie. Acta Neuropathol 1987; 73: 379-382.

78. Giese A, Brown DR, Groschup MH, Feldmann C, Haist I, Kretzschmar HA. Role of microglia in neuronal cell death in prion disease. Brain Pathol 1998; 8: 449-457.

79. Giese A, Groschup MH, Hess B, Kretzschmar HA. Neuronal cell death in scrapie-infected mice is due to apoptosis. Brain Pathol 1995; 5: 213-221.

80. Giese A, Kretzschmar HA. Prion-induced neuronal damage – the mechanisms of neuronal destruction in the subacute spongiform encephalopathies. Curr Top Microbiol Immunol 2001; 253: 203-217.

81. Glatzel M, Giger O, Braun N, Aguzzi A. The peripheral nervous system and the pathogenesis of prion diseases. Curr Mol Med 2004; 4: 355-359.

82. Gonzalez-Polo RA, Boya P, Pauleau AL, Jalil A, Larochette N, Souquere S, Eskelinen EL, Pierron G, Saftig P, Kroemer G. The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 2005; 118: 3091-3102.

83. Graeber MB, Moran LB. Mechanisms of cell death in neurodegenerative diseases: fashion, fiction, and facts. Brain Pathol 2002; 12: 385-390.

84. Gray F, Chretien F, Adle-Biassette H, Dorandeu A, Ereau T, Delisle M-B, Kopp N, Ironside JW, Vital C. Neuronal apoptosis in Creutzfeldt-Jakob disease. J Neuropathol Exp Neurol 1999; 58: 321-328.

85. Griffith JS. Self-replication and scrapie. Nature 1967; 215: 1043-1044.

86. Guan Z, Soderberg M, Sindelar P, Prusiner SB, Kristensson K, Dallner G. Lipid composition in scrapie-infected mouse brain: prion infection increases the levels of dolichyl phosphate and ubiquinone. J Neurochem 1996; 66: 277-285.

87. Guentchev M, Voigtländer T, Haberler C, Groschup MH, Budka H. Evidence for oxidative stress in experimental prion disease. Neurobiol Dis 2000; 7: 270-273.

88. Hafiz FB, Brown DR. A model for the mechanism of astrogliosis in prion disease. Mol Cell Neurosci 2000; 16: 221-232.

89. Hainfellner H, Liberski PP, Guiroy DC, Cervenakova L, Brown P, Gajdusek DC, Budka H. Pathology and immunohistochemistry of a Kuru brain. Brain Pathol 1997; 7: 547-554.

90. Hardy J, Scholz S, Evans W, Goldfarb L, Singleton A. Prion genotypes in Central America suggest selection for the V129 allele. Am J Med Genet B Neuropsychiatr Genet 2006; 141: 33-35.

91. Hariri M, Millane G, Guimond M-P, Dennis JW, Nabi IR. Biogenesis of multilamellar bodies via autophagy. Mol Biol Cell 2000; 11: 255-268.

92. Harris DA. Trafficking, turnover and membrane topology of PrP. Br Med Bull 2003; 66: 71-85.

93. Harris DA, Falls DL, Johnson FA, Fischbach GD. A prion-like protein from chicken brain copurifies with an acetylcholine receptor-inducing activity. Proc Natl Acad Sci USA 1991; 88: 7664-7668.

94. Hedge RS, Mastrianni JA, Scott MR, DeFea KA, Tremblay P, Torchia M, DeArmond SJ, Prusiner SB, Lingappa VR. A transmembrane form of the prion protein in neurodegenerative disease. Science 1998; 279: 827-834.

95. Hegde RS, Voigt S, Lingappa VR. Regulation of protein topology by trans-acting factors at the endoplasmic reticulum. Mol Cell 1998; 2: 85-91.

96. Hetz C, Russelakis-Carneiro M, Maundrell K, Castilla J, Soto C. Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. EMBO J 2003; 22: 5435-5445.

97. Hijazi N, Kariv-Inbal Z, Gasset M, Gabizon R. PrPSc incorporation to cells requires endogenous glycosaminoglycan expression. J Biol Chem 2005; 280: 17057-17061.

98. Horonchik L, Tzaban S, Ben Zaken O, Yedidia Y, Rouvinski A, Papy-Garcia D, Barritault D, Vlodavsky I, Taraboulos A. Heparan sulfate is a cellular receptor for purified infectious prions. J Biol Chem 2005; 280: 17062-17067.

99. Inbal B, Bialik S, Sabanay I, Shani G, Kimchi A. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol 2002; 157: 455-468.

100. Jamieson E, Jeffrey M, Ironside JW, Fraser JR. Apoptosis and dendritic dysfunction precede prion protein accumulation in 87V scrapie. Neuroreport 2001; 12: 2147-2153.

101. Jeffrey M, Fraser JR, Halliday WG, Fowler N, Goodsir CM, Brown DA. Early unsuspected neuron and axon terminal loss in scrapie-infected mice revealed by morphometry and immunohisto-chemistry. Neuropathol Appl Neurobiol 1995; 21: 41-49.

102. Jeffrey M, Goodbrand IA, Goodsir A. Pathology of the transmissible spongiform encephalopathies with special emphasis on ultrastructure. Micron 1995; 26: 277-298.

103. Jeffrey M, Goodsir CM, Race RE, Chesebro B. Scrapie-specific neuronal lesions are independent of neuronal PrP expression. Ann Neurol 2004; 55: 781-792.

104. Jeffrey M, McGovern G, Goodsir CM, Brown KL, Bruce ME. Sites of prion protein accumulation in scrapie-infected mouse spleen revealed by immuno-electron microscopy. J Pathol 2000; 191: 323-332.

105. Jeffrey M, Scott JR, Williams A, Fraser H. Ultrastructural features of spongiform encephalopathy transmitted to mice from three species of bovidae. Acta Neuropathol 1992; 84: 559-569.

106. Jeffrey M, Wells GA. Spongiform encephalopathy in a Nyala. Vet Pathol 1988; 25: 398-399.

107. Jesionek-Kupnicka D, Buczynski J, Kordek R, Liberski PP. Neuronal loss and apoptosis in experimental Creutzfeldt-Jakob disease in mice. Folia Neuropathol 1999; 37: 283-286.

108. Jesionek-Kupnicka D, Buczynski J, Kordek R, Sobow T, Kloszewska I, Papierz W, Liberski PP. Programmed cell death (apoptosis) in Alzheimer's disease and Creutzfeldt-Jakob disease. Folia Neuropathol 1997; 35: 233-235.

109. Jesionek-Kupnicka D, Kordek R, Buczynski J, Liberski PP. Apoptosis in relation to neuronal loss in experimental Creutzfeldt-Jakob disease in mice. Acta Neurobiol Exp (Wars) 2001; 61: 13-19.

110. Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, DiFiglia M. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. J Neurosci 2000; 20: 7268-7278.

111. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-257.

112. Kim JI, Ju WK, Choi JH, Choi E, Carp RI, Wisniewski HM, Kim YS. Expression of cytokine genes and increased nuclear factor-kappa B activity in the brains of scrapie-infected mice. Brain Res Mol Brain Res 1999; 73: 17-27.

113. Kimberlin RH. Scrapie and possible relationships with viroids. Sem Virol 1999; 1: 153-162.

114. Kimberlin RH, Field HJ, Walker CA. Pathogenesis of mouse scrapie: evidence for spread of infection from central to peripheral nervous system. J Gen Virol 1983; 64: 713-716.

115. Kirkwood JK, Wells GAH, Cunningham AA, Jackson SI, Scott AC, Dawson M, Wilesmith JW. Scrapie-like encephalopathy in a greater kudu (Tragelaphus strepsiceros) which had not been fed ruminant-derived protein. Vet Res 1992; 130: 365-367.

116. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000; 290: 1717-1721.

117. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 2000; 10: 524-530.

118. Kovacs GG, Preusser M, Strohschneider M, Budka H. Subcellular localization of disease-associated prion protein in the human brain. Am J Pathol 2005; 166: 287-294.

119. Kretzschmar HA, Giese A, Brown DR, Herms J, Keller B, Schmidt B, Groschup MH. Cell death in prion disease. J Neural Transm Suppl 1997; 50: 191-210.

120. Kretzschmar HA, Giese A, Brown DR, Herms J, Schmidt B, Groschup MH. Cell death in prion disease. In: Court L, Dodet B (eds.). Transmissible Subacute Spongiform Encephalopathies: Prion Disease. IIIrd International Symposium on transmissible subacute spongiform encephalopathies: prion disease. Val-de-Grace, Paris, France. Elsevier, Amsterdam-Oxford-Paris, 1996; pp. 97-106.

121. Kretzschmar HA, Giese A, Herms J, Brown DR. Neuronal degeneration and cell death in prion disease. In: Morrison DR (ed.). Prions and Brain Diseases in Animals and Humans. Plenum Press, New York, 1998; pp. 253-268.

122. Kristiansen M, Messenger MJ, Klöhn PC, Brandner S, Wadsworth JD, Collinge J, Tabrizi SJ. Disease-related prion protein forms aggresomes in neuronal cells leading to caspase activation and apoptosis. J Biol Chem 2005; 280: 38851-38861.

123. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P, Zhivotovsky B, Blagosklonny MV, Malorni W, Knight RA, Piacentini M, Nagata S, Melino G. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ 2005; 12 (Suppl 2): 1463-1467.

124. Kroemer G, Jaattela M. Lysosomes and autophagy in cell death control. Nat Rev Cancer 2005; 5: 886-897.

125. Künzi V, Glatzel M, Nakano MY, Greber UF, Van Leuven F, Aguzzi A. Unhampered prion neuroinvasion despite impaired fast axonal transport in transgenic mice overexpressing four-repeat tau. J Neurosci 2002; 22: 7471-7477.

126. Kuwahara C, Takeuchi AM, Nishimura T, Haraguchi K, Kubosaki A, Matsumoto Y, Saeki K, Matsumoto Y, Yokoyama T, Itohara S, Onodera T. Prions prevent neuronal cell-line death. Nature 1999; 400: 225-226.

127. Lasmezas CI, Deslys JP, Robain O, Jaegly A, Beringue V, Peyrin JM, Fournier JG, Hauw JJ, Rossier J, Dormont D. Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. Science 1997; 275: 402-405.

128. Lee DW, Sohn HO, Lim HB, Lee YG, Kim Y-S, Carp RI, Wisniewski HM. Alteration of free radical metabolism in the brain of mice infected with scrapie agent. Free Radic Res 1999; 30: 499-507.

129. Lee HP, Jun YC, Choi JK, Kim JI, Carp RI, Kim YS. Activation of mitogen-activated protein kinases in hamster brains infected with 263K scrapie agent. J Neurochem 2005; 95: 584-593.

130. Levine P. Scrapie: an infective polypeptide? Lancet 1972; 1: 748.

131. Liberski PP. Electron microscopic observations on dystrophic neurites in hamster brains infected with the 263K strain of scrapie. J Comp Pathol 1987; 97: 35-39.

132. Liberski PP, Asher DM, Yanagihara R, Gibbs CJ Jr, Gajdusek DC. Serial ultrastructural studies of scrapie in hamsters. J Comp Pathol 1989; 101: 429-442.

133. Liberski PP, Budka H. Neuroaxonal pathology in Creutzfeldt-Jakob disease. Acta Neuropathol 1999; 97: 329-334.

134. Liberski PP, Budka H, Yanagihara R, Gajdusek DC. Neuroaxonal dystrophy in experimental Creutzfeldt-Jakob disease: electron microscopical and immunohistochemical demonstration of neurofilament accumulations within affected neurites. J Comp Pathol 1995; 112: 243-255.

135. Liberski PP, Gajdusek DC. Kuru: forty years later. Brain Pathol 1997; 7: 555-560.

136. Liberski PP, Gajdusek DC, Brown P. How do neurons degenerate in prion diseases or transmissible spongiform encephalopathies (TSEs): neuronal autophagy revisited. Acta Neurobiol Exp (Wars) 2002; 62: 141-147.

137. Liberski PP, Guiroy DC, Williams ES, Walis A, Budka H. Deposition patterns of disease-associated prion protein in captive mule deer brains with chronic wasting disease. Acta Neuropathol 2001; 102: 496-500.

138. Liberski PP, Ironside JW. An outline of the neuropathology of transmissible spongiform encephalopathies (prion diseases). Folia Neuropathol 2004; 42 (Suppl B): 39-58.

139. Liberski PP, Jaskolski M. Prion diseases: a dual view of the prion hypothesis as seen from a distance. Acta Neurobiol Exp (Wars) 2002; 62: 197-224.

140. Liberski PP, Jeffrey M. Tubulovesicular structures – the ultrastructural hallmark for transmissible spongiform encephalopathies or prion diseases. Folia Neuropathol 2004; 42 (Suppl B): 96-108.

141. Liberski PP, Kloszewska I, Boellaard I, Papierz W, Omulecka A, Budka H. Dystrophic neurites of Alzheimer's disease and Gerstmann-Sträussler-Scheinker's disease dissociate from the formation of paired helical filaments. Alzheimer's Res 1995; 1: 89-93.

142. Liberski PP, Mori S. The Echigo-1: a panencephalopathic strain of Creutzfeldt-Jakob disease: a passage to hamsters and ultrastructural studies. Folia Neuropathol 1997; 35: 250-254.

143. Liberski PP, Sikorska B, Bratosiewicz-Wasik J, Gajdusek DC, Brown P. Neuronal cell death in transmissible spongiform encephalopathies (prion diseases) revisited: from apoptosis to autophagy. Int J Biochem Cell Biol 2004; 36: 2473-2490.

144. Liberski PP, Streichenberger N, Giraud P, Soutrenon M, Meyronnet D, Sikorska B, Kopp N. Ultrastructural pathology of prion diseases revisited: brain biopsy studies. Neuropathol Appl Neurobiol 2005; 31: 88-96.

145. Liberski PP, Yanagihara R, Gibbs CJ Jr, Gajdusek DC. Neuronal autophagic vacuoles in experimental scrapie and Creutzfeldt-Jakob disease. Acta Neuropathol 1992; 83: 134-139.

146. Lucassen PJ, Williams A, Chung WC, Fraser H. Detection of apoptosis in murine scrapie. Neurosci Lett 1995; 198: 185-188.

147. Lugaresi E, Medori R, Montagna P, Baruzzi A, Cortelli P, Lugaresi A, Tinuper P, Zucconi M, Gambetti P. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. New Engl J Med 1986; 315: 997-1003.

148. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005; 120: 237-248.

149. Magalhaes AC, Baron GS, Lee KS, Steele-Mortimer O, Dorward D, Prado MA, Caughey B. Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells. J Neurosci 2005; 25: 5207-5216.

150. Magalhaes AC, Silva JA, Lee KS, Martins VR, Prado VF, Ferguson SS, Gomez MV, Brentani RR, Prado MA. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J Biol Chem 2002; 277: 33311-33318.

151. Mallucci G, Dickinson A, Linehan J, Klohn PC, Brandner S, Collinge J. Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. Science 2003; 302: 871-874.

152. Manuelidis L. A virus behind the mask of prions? Folia Neuropathol 2004; 42 (Suppl B): 10-23.

153. Manuelidis L. A 25 nm virion is the likely cause of transmissible spongiform encephalopathies. J Cell Biochem 2007; 100: 897-915.

154. Manuelidis L, Sklaviadis T, Manuelidis EE. Evidence suggesting that PrP is not the infectious agent in Creutzfeldt-Jakob disease. EMBO J 1987; 6: 341-347.

155. Marella M, Chabry J. Neurons and astrocytes respond to prion infection by inducing microglia recruitment. J Neurosci 2004; 24: 620-627.

156. Marella M, Gaggioli C, Batoz M, Deckert M, Tartare-Deckert S, Chabry J. Pathological prion protein exposure switches on neuronal mitogen-activated protein kinase pathway resulting in microglia recruitment. J Biol Chem 2005; 280: 1529-1534.

157. Masters CL, Gajdusek DC, Gibbs CJ Jr. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome. With an analysis of the various forms of amyloid plaque deposition in the virus induced spongiform encephalopathies. Brain 1981; 104: 559-588.

158. Matsumura H, Shimizu Y, Ohsawa Y, Kawahara A, Uchiyama Y, Nagata S. Necrotic death pathway in Fas receptor signaling. J Cell Biol 2000; 151: 1247-1256.

159. McBride PA, Schulz-Schaeffer WJ, Donaldson M, Bruce M, Diringer H, Kretzschmar HA, Beekes M. Early spread of scrapie from the gastrointestinal tract to the central nervous system involves autonomic fibers of the splanchnic and vagus nerves. J Virol 2001; 75: 9320-9327.

160. McHattie SJ, Brown DR, Bird MM. Cellular uptake of the prion protein fragment PrP106-126 in vitro. J Neurocytol 1999; 28: 149-159.

161. McKinley MP, Bolton DC, Prusiner SB. A protease resistant protein is a structural component of the scrapie prion. Cell 1983; 35: 57-62.

162. Melino G, Knight RA, Nicotera P. How many ways to die? How many different models of cell death? Cell Death Differ 2005; 12 (Suppl 2): 1457-1462.

163. Merz PA, Rohwer RG, Kascsak R, Wisniewski HM, Somerville RA, Gibbs CJ Jr, Gajdusek DC. Infection specific particle from the unconventional slow virus diseases. Science 1984; 225: 437-440.

164. Merz PA, Somerville RA, Wisniewski HM, Iqbal K. Abnormal fibrils from scrapie-infected brain. Acta Neuropathol 1981; 54: 63-74.

165. Migheli A, Attanasio A, Lee WH, Bayer SA, Ghetti B. Detection of apoptosis in weaver cerebellum by electron microscopic in situ end-labeling of fragmented DNA. Neurosci Lett 1995; 199: 53-56.

166. Millson GC, Hunter GD, Kimberlin RH. The physico-chemical nature of the scrapie agent. In: Kimberlin RH (ed.). Slow Virus Diseases of Animals and Man. North-Holland Publ Comp, Amsterdam, 1976; pp. 243-266.

167. Mishra RS, Basu S, Gu Y, Luo X, Zou WQ, Mishra R, Li R, Chen SG, Gambetti P, Fujioka H, Singh N. Protease-resistant human prion protein and ferritin are cotransported across Caco-2 epithelial cells: implications for species barrier in prion uptake from the intestine. J Neurosci 2004; 24: 11280-11290.

168. Moran LB, Kosel S, Spitzer C, Schwaiger FW, Riess O, Kreutzberg GW, Graeber MB. Expression of alpha-synuclein in non-apoptotic, slowly degenerating facial motoneurones. J Neurocytol 2001; 30: 515-521.

169. Morel E, Andrieu T, Casagrande F, Gauczynski S, Weiss S, Grassi J, Rousset M, Dormont D, Chambaz J. Bovine prion is endocytosed by human enterocytes via the 37 kDa/67 kDa laminin receptor. Am J Pathol 2005; 167: 1033-1042.

170. Moya KL, Hässig R, Breen KC, Volland H, Di Giamberardino L. Axonal transport of the cellular prion protein is increased during axon regeneration. J Neurochem 2005; 92: 1044-1053.

171. Moya KL, Hässig R, Créminon C, Laffont I, Di Giamberardino L. Enhanced detection and retrograde axonal transport of PrPc in peripheral nerve. J Neurochem 2004; 88: 155-160.

172. Muller WE, Ushijima H, Schroder HC, Forrest JM, Schatton WF, Rytik PG, Heffner-Lauc M. Cytoprotective effect of NMDA receptor antagonists on prion protein (PrionSc)-induced toxicity in rat cortical cell cultures. Eur J Pharmacol 1993; 246: 261-267.

173. Myohara M. Real-time observation of autophagic programmed cell death of Drosophila salivary glands in vitro. Dev Genes Evol 2004; 214: 99-104.

174. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol 2005; 64: 113-122.

175. O'Donovan CN, Tobin D, Cotter TG. Prion protein fragment PrP-(106-126) induces apoptosis via mitochondrial disruption in human neuronal SH-SY5Y cells. J Biol Chem 2001; 276: 43516-43523.

176. Oesch B, Westaway D, Wälchli M, McKinley MP, Kent SB, Aebersold R, Barry RA, Tempst P, Teplow DB, Hood LE, et al. A cellular gene encodes scrapie PrP 27-30 protein. Cell 1985; 40: 735-746.

177. Oidtmann B, Simon D, Holtkamp N, Hoffmann R, Baier M. Identification of cDNAs from Japanese pufferfish (Fugu rubripes) and Atlantic salmon (Salmo salar) coding for homologues to tetrapod prion proteins. FEBS Lett 2003; 538: 96-100.

178. Peretz D, Williamson RA, Kaneko K, Vergara J, Leclerc E, Schmitt-Ulms G, Mehlhorn IR, Legname G, Wormald MR, Rudd PM, Dwek RA, Burton DR, Prusiner SB. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. Nature 2001; 412: 739-743.

179. Pillot T, Drouet B, Pinçon-Raymond M, Vandekerckhove J, Rosseneu M, Chambaz J. A nonfibrillar from of the fusogenic prion protein fragment (118-135) induces apoptotic cell death in rat cortical neurons. J Neurochem 2000; 75: 2298-2308.

180. Prado MA, Alves-Silva J, Magalhaes AC, Prado VF, Linden R, Martins VR, Brentani RR. PrPc on the road: trafficking of the cellular prion protein. J Neurochem 2004; 88: 769-781.

181. Prusiner SB. Novel proteinaceous infection particles cause scrapie. Science 1982; 216: 136-144.

182. Prusiner SB. Terminology. In: Prusiner SB, McKinley MP (eds.). Prions. Novel Infectious Pathogens Causing Scrapie and Creutzfeldt-Jakob Disease. Academic Press, New York, 1987; pp. 38-53.

183. Prusiner SB. Shattuck lecture – neurodegenerative diseases and prions. N Engl J Med 2001; 344: 1516-1526.

184. Prusiner SB (ed.). Prion Biology and Diseases. University of California, San Francisco, 2004.

185. Prusiner SB, McKinley MP, Bowman KA, Bolton DC, Bendheim PE, Groth DF, Glenner GG. Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 1983; 35: 349-358.

186. Race R, Oldstone M, Chesebro B. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of PrP expression in peripheral nerves and spleen. J Virol 2000; 74: 828-833.

187. Raeber AJ, Race RE, Brandner S, Priola SA, Sailer A, Bessen RA, Mucke L, Manson J, Aguzzi A, Oldstone MB, Weissmann C, Chesebro B. Astrocyte-specific expression of hamster prion protein (PrP) renders PrP knockout mice susceptible to hamster scrapie. EMBO J 1997; 16: 6057-6065.

188. Raina AK, Zhu X, Smith MA. Alzheimer's disease and the cell cycle. Acta Neurobiol Exp (Wars) 2004; 64: 107-112.

189. Reggiori F, Klionsky DJ. Autophagy in the eukaryotic cell. Eukaryot Cell 2002; 1: 11-21.

190. Rieger R, Edenhofer F, Lasmezas CI, Weiss S. The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells. Nat Med 1997; 3: 1383-1388.

191. Saez-Valero J, Angeretti N, Forloni G. Caspase-3 activation by beta-amyloid and prion protein peptides is independent from their neurotoxic effect. Neurosci Lett 2000; 293: 207-210.

192. Sattler T, Mayer A. Cell-free reconstitution of microautophagic vacuole invagination and vesicle formation. J Cell Biol 2000; 151: 529-538.

193. Schatzl HM, Da Costa M, Taylor L, Cohen FE, Prusiner SB. Prion protein gene variation among primates. J Mol Biol 1995; 45: 362-374.

194. Schatzl HM, Da Costa M, Taylor L, Cohen FE, Prusiner SB. Prion protein gene variation among primates. J Mol Biol 1997; 265: 257.

195. Schatzl HM, Laszlo L, Holtzman DM, Tatzelt J, DeArmond SJ, Weiner RI, Mobley WC, Prusiner SB. A hypothalamic neuronal cell line persistently infected with scrapie prions exhibits apoptosis. J Virol 1997; 71: 8821-8831.

196. Schulz-Schaeffer WJ, Tschoke S, Kranefuss N, Drose W, Hause-Reitner D, Giese A, Groschup MH, Kretzschmar HA. The paraffin-embedded tissue blot detects PrP(Sc) early in the incubation time in prion diseases. Am J Pathol 2000; 156: 51-56.

197. Schweichel JU, Merker HJ. The morphology of various types of cell death in prenatal tissues. Teratology 1973; 7: 253-266.

198. Shyng S-L, Huber MT, Harris DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J Biol Chem 1993; 268: 15922-15928.

199. Sikorska B, Liberski PP, Giraud P, Kopp N, Brown P. Autophagy is a part of ultrastructural synaptic pathology in Creutzfeldt-Jakob disease: a brain biopsy study. Int J Biochem Cell Biol 2004; 36: 2563-2573.

200. Simonic T, Duga S, Strumbo B, Asselta R. cDNA cloning of turtle prion protein. FEBS Lett 2000; 469: 33-38.

201. Siso S, Puig B, Varea R, Vidal E, Acin C, Prinz M, Montrasio F, Badiola J, Aguzzi A, Pumarola M, Ferrer I. Abnormal synaptic protein expression and cell death in murine scrapie. Acta Neuropathol 2002; 103: 615-626.

202. Soldevila M, Andres AM, Ramirez-Soriano A, Marques-Bonet T, Calafell F, Navarro A, Bertranpetit J. The prion protein gene in humans revisited: lessons from a worldwide resequencing study. Genome Res 2006; 16: 231-239.

203. Solforosi L, Criado JR, McGavern DB, Wirz S, Sanchez-Alavez M, Sugama S, DeGiorgio LA, Volpe BT, Wiseman E, Abalos G, Masliah E, Gilden D, Oldstone MB, Conti B, Williamson RA. Cross-linking cellular prion protein triggers neuronal apoptosis in vivo. Science 2004; 303: 1514-1516.

204. Sparkes RS, Simon M, Cohn VH, Fournier RE, Lem J, Klisak I, Heinzmann C, Blatt C, Lucero M, Mohandas T, et al. Assignment of the human and mouse prion protein genes to homologous chromosomes. Proc Natl Acad Sci USA 1986; 83: 7358-7362.

205. Sperandio S, de Belle I, Bredesen DE. An alternative, nonapoptotic form of programmed cell death. Proc Natl Acad Sci USA 2000; 97: 14376-14381.

206. Stadelmann C, Deckwerth TL, Srinivasan A, Bancher C, Bruck W, Jellinger K, Lassmann H. Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death. Am J Pathol 1999; 155: 1459-1466.

207. Stahl N, Baldwin MA, Hecker R, Pan K-M, Burlingame AL, Prusiner SB. Glycosylinsoitol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. Biochemistry 1992; 31: 5043-5053.

208. Stahl N, Borchelt DR, Prusiner SB. Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C. Biochemistry 1990; 29: 5405-5412.

209. Strumbo B, Ronchi S, Bolis LC, Simonic T. Molecular cloning of the cDNA coding for Xenopus laevis prion. FEBS Lett 2001; 508: 170-174.

210. Stumpf MP, Krakauer DC. Mapping the parameters of prion-induced neuropathology. Proc Natl Acad Sci USA 2000; 97: 10573-10577.

211. Tarabulos A, Jendroska K, Serban D, Yang S-L, DeArmond SJ, Prusiner SB (1992) Regional mapping of prion protein in brain. Proc Natl Acad Sci USA 89: 7620-7624.

212. Theil D, Fatzer R, Meyer R, Schobesberger M, Zurbriggen A, Vandevelde M. Nuclear DNA fragmentation and immune reactivity in bovine spongiform encephalopathy. J Comp Pathol 1999; 121: 357-367.

213. Thellung S, Florio T, Villa V, Corsaro A, Arena S, Amico C, Robello M, Salmona M, Forloni G, Bugiani O, Tagliavini F, Schettini G. Apoptotic cell death and impairment of L-type voltage-sensitive calcium channel activity in rat cerebellar granule cells treated with the prion protein fragment 106-126. Neurobiol Dis 2000; 7: 299-309.

214. van Rheede T, Smolenaars MM, Madsen O, de Jong WW. Molecular evolution of the mammalian prion protein. Mol Biol Evol 2003; 20: 111-121.

215. Wang C-W, Klionsky DJ. The molecular mechanism of autophagy. Mol Med 2003; 9: 65-76.

216. Weissmann C. A "unified theory" of prion propagation. Nature 2000; 352: 679-683.

217. Weissmann C. The state of the prion. Nat Rev Microbiol 2004; 2: 861-871.

218. Wells GA, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R. A novel progressive spongiform encephalopathy in cattle. Vet Rec 1987; 121: 419-420.

219. White AR, Collins SJ, Maher F, Jobling MF, Stewart LR, Thyer JM, Beyreuther K, Masters CL, Cappai R. Prion protein-deficient neurons reveal lower glutathione reductase activity and increased susceptibility to hydrogen peroxide toxicity. Am J Pathol 1999; 155: 1723-1730.

220. Williams A, Lucassen PJ, Ritchie D, Bruce M. PrP deposition, microglial activation, and neuronal apoptosis in murine scrapie. Exp Neurol 1997; 144: 433-438.

221. Williams ES, Young S. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J Wildl Dis 1980; 16: 89-98.

222. Williams ES, Young S. Spongiform encephalopathy of Rocky Mountain elk. J Wildl Dis 1982; 18: 465-471.

223. Willoughby K, Kelly DF, Lyon DG, Wells GA. Spongiform encephalopathy in a captive puma (Felis concolor). Vet Rec 1992; 131: 431-434.

224. Wood JL, Lund LJ, Done SH. The natural occurrence of scrapie in moufflon. Vet Rec 1992; 130: 25-27.

225. Wopfner F, Weidenhofer G, Schneider R, von Brunn A, Gilch S, Schwarz TF, Werner T, Schatzl HM. Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. J Mol Biol 1999; 289: 1163-1178.

226. Wyatt JM, Pearson GR, Smerdon TN, Gruffydd-Jones TJ, Wells GA, Wilesmith JW. Naturally occurring scrapie-like spongiform encephalopathy in five domestic cats. Vet Rec 1991; 129: 233-236.

227. Xue L, Fletcher GC, Tolkovsky AM. Autophagy is activated by apoptotic signalling in sympathetic neurons: an alternative mechanism of death execution. Mol Cell Neurosci 1999; 14: 180-198.

228. Yakovlev AG, Faden AI. Mechanisms of neural cell death: implications for development of neuroprotective treatment strategies. NeuroRx 2004; 1: 5-16.

229. Ye X, Scallet AC, Kascsak RJ, Carp RI. Astrocytosis and amyloid deposition in scrapie-infected hamsters. Brain Res 1998; 809: 277-287.

230. Yuan J, Lipinski M, Degterev A. Diversity of the mechanisms of neuronal cell death. Neuron 2003; 40: 401-413.

231. Zanata SM, Lopes MH, Mercadante AF, Hajj GN, Chiarini LB, Nomizo R, Freitas AR, Cabral AL, Lee KS, Juliano MA, de Oliveira E, Jachieri SG, Burlingame A, Huang L, Linden R, Brentani RR, Martins VR. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. EMBO J 2002; 21: 3307-3316.