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REVIEW ARTICLE

Prion biology and bovine spongiform encephalopathy

Biología del prion y encefalopatía espongiforme bovina

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RESUMEN

La compleja naturaleza de los priones ha intrigado a la comunidad científica durante los últimos 70 años. Desde el primer hallazgo de la infectividad del scrapie y la primera transmisión experimental de este agente en 1937, los priones y las encefalopatías espongiformes transmisibles (EETs) han estado bajo intensa investigación. Las EETs son enfermedades neurodegenerativas y fatales que no poseen diagnóstico temprano, tratamiento o cura. A pesar de sus diversas presentaciones, estas enfermedades provienen de la conversión infecciosa, espontánea o hereditaria de la proteína del prion celular PrP^{C} en una isoforma patogénica conocida como PrP^{Sc}. Según la hipótesis del prion, la PrP^{C} tiene la capacidad autocatalítica o inducida de cambiar su configuración secundaria desde una estructura α-helicoidal a una configuración predominantemente β-planar, característica de la PrP^{Sc}. La potencial función fisiológica de la PrP^{C} aún no ha sido determinada, sin embargo ha sido vinculada a varios roles biológicos que incluyen adhesión, protección y diferenciación celular. La naturaleza compleja de las EETs ha abierto nuevas interrogantes y presenta nuevos desafios en el área de la biología del prion. La potencial transmisión zoonótica de la Encefalopatía Espongiforme Bovina (EEB) ha despertado preocupación en la comunidad internacional en relación a la bioseguridad de los productos de origen animal. Como consecuencia de este fenómeno, se han realizado numerosas investigaciones para establecer la patogénesis de las EETs y desarrollar métodos de diagnóstico y tratamiento. Sin embargo, se requiere de mayores esfuerzos para dilucidar la compleja naturaleza de este agente y establecer métodos de control para estas enfermedades.

Key words: Pathogenic prion protein (PrP^{Sc}), Bovine spongiform encephalopathy (BSE).

Palabras clave: Proteína patogénica del prion (PrP^{Sc}), Encefalopatía espongiforme bovina (EEB).

INTRODUCTION

The scientific community has been intrigued with the complex nature of prions for over 70 years. The first indication of prion infectivity was reported in 1937 in Scotland after immunization of sheep against louping ill. The vaccines used were accidentally elaborated from brain extracts obtained from sheep infected with scrapie (Gordon 1946). The fact that eight percent of the immunized animals developed scrapie, with the experimental transmission of the agent performed the same year, demonstrated the infectious capacity of scrapie among sheep and goats (Cullie and Chele 1938). In 1959, Hadlow suggested that Kuru, a neurodegenerative disease that affected New Guinea tribes might be similar to scrapie due to similarities in epidemiology, clinical signs and pathological findings. This hypothesis was later confirmed in 1965 by the successful transmission of Kuru to chimpanzees after incubation of 18 to 21 months (Gajdusek *et al* 1966). One year later, Alper and colleagues reported that the molecular weight of the scrapie agent was significantly lower compared to a conventional virus (Alper *et al* 1966). Moreover, Apler showed that the scrapie agent was able to resist doses of ultraviolet radiation (UV) that are sufficient to inactivate nucleic acids. These experiments led to the formulation of the protein-only hypothesis, which described the scrapie agent as a particle conformed by a proteinaceous structure devoid of nucleic acids with the unique capacity to autoreplicate (Griffith 1967). In the 1980s, Prusiner reported abundant experimental data in support of this hypothesis and proposed for the first time the term "prion" to describe the scrapie agent. A prion was defined as small proteinaceous infectious particle which was resistant to inactivation by most procedures that modify nucleic acids (Prusiner 1982). This controversial suggestion supported the idea of a scrapie agent consisting only of an infectious protein and discredited the model that included a small nucleic acid in the core of the protein. Furthermore, one of the most intriguing aspects of the prion biology was the discovery of a host-encoded cellular prion protein or PrP^{C} (Oesch *et al* 1985). This discovery guided to the formulation of the prion hypothesis that postulates that the agent responsible for prion propagation is originated by autocatalytic conversion of PrP^{C} into the pathogenic isoform PrP^{Sc}.

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The unpredictable properties of PrP^{Sc} and the potential zoonotic transmission of the bovine spongiform encephalopathy (BSE) have generated intense concern in the international community over animal product biosecurity. Worldwide, 214 people have fallen victim to the variant CJD (vCJD) until January 2011, and epidemiologists predict the presentation of more cases in the coming years due to existing pre-clinical or subclinical infection (Collee *et al* 2006, NCJDSU[1]). In USA, the first case of BSE was detected in 2003 resulting in the ban of meat importation to 53 countries with a drastic drop in 83% of the imports. Translated into economic terms, BSE produced a loss of 5 billion dollars which corresponded to 4% of the gross domestic agriculture product (Coffey *et al* 2005). The complex presentation of Transmissible Spongiform Encephalopathies (TSEs) and the novel properties of the PrP^{Sc} have opened many questions yet to be answered. The objective of this report is to present an update of the current knowledge on prion biology and to review some of the features of BSE pathophysiology. During the last years, research in prion biology has mainly focused on determination of the pathogenesis of TSEs and the development of diagnostic and therapeutic methods. However, further research in prion biology will continue to be the foundation for understanding the complex nature of TSEs and how these diseases can be controlled.

PRION BIOLOGY

PrP^{C} STRUCTURE

The structure of PrP^{C} is highly conserved among species and throughout evolution, suggesting an important biological role (Gossert *et al* 2005). Before post-translational modification, PrP^{C} is composed of a sequence of 253 amino acids with a slight variation between species depending on the number of octapeptide repeats (Prusiner and Scott 1997). The octapeptide repeat region is an eight-amino acid repetitive motif composed of residues P(H/Q) GGG(-/G)WGQ and located in the N-terminal region of the protein (Moore *et al* 2006). During protein maturation, PrP^{C} is exposed to several modifications in the rough endoplasmic reticulum (ER) including replacement of the peptide signal located between amino acids 232-253 with a glycophosphatidylinositol (GPI) anchor. Additionally, two asparagines at amino acids 181-197 are glycosylated and one disulfide bridge is added between two cysteine residues 179-214 (Prusiner 1998, Harris 2003). The mature protein is divided in two distinct regions: a flexible and essentially unstructured N-terminal region between amino acids 23-125 and a C-terminal region containing three alpha-helicoidal structures and a short beta-sheet motif between amino acids 126-231 (Abid and Soto 2006). Asparagine glycosylation will determine variations in the biochemistry of the mature protein resulting in di-, mono-, or un-glycosylated forms with molecular weights of ~34, 28, and 25 Kb, respectively (Russelakis-Carneiro *et al* 2002). PrP^{C} is found as a mixture of these forms with variable proportions depending on the tissue and animal species (Russelakis-Carneiro *et al* 2002). The expression of PrP^{C} is high in the central nervous system; however, recent reports have showed a wide expression in various somatic tissues including thymus, intestine, heart, kidney and skin (Peralta and Eyestone 2009).

The usual cellular location of PrP^{C} is attached by the GPI anchor to membrane domains rich in cholesterol and sphingolipids known as lipid raft (Martins *et al* 2002, DeMarco and Daggett 2005). However, part of the pool of PrP^{C} can be internalized via clathrin-mediated endocytosis and accumulate inside the Golgi apparatus. Furthermore, some of the internalized protein is recycled to the cytoplasmatic membrane by kinesin anterograde transport (Hachiya *et al* 2004). Although the specific location for PrP^{Sc} conversion has not yet been determined, it is believed that formation of PrP^{Sc} occurs at the exterior face of the plasma membrane (Jeffrey *et al* 2011). Endocytosis of PrP^{Sc} may occur mediated by ubiquitin and clathrin molecules into lysosomes for degradation (Jeffrey *et al* 2011).

PRION GENE STRUCTURE

The prion gene (*PRNP*) has homologues in all vertebrates with conserved regions between mammals and birds (Premzl and Gamulin 2007). *PRNP* is located in chromosome 2 in mouse, 13 in bovine and 20 in human (Sparkes *et al* 1986, Ryan and Womack 1993). The 5' flanking region of bovine *PRNP* shows an 89% homology with the sheep and only 46-62% homology with the mouse, rat, hamster and human sequences (Inoue *et al* 1997). Bovine, sheep, mouse and rat *PRNP* possess three exons with the protein coding sequence located entirely within the third exon (figure 1) (Oesch *et al* 1985, Inoue *et al* 1997). Using chloramphenicol acetyltransferase (CAT) plasmids, the promoter region of the bovine gene was detected in the region between –88 and –30 relative to the transcription start site, similar to the rat promoter region (Inoue *et al* 1997). Several regulatory regions including the promoter has been identified in the bovine *PRNP* with the major region of transcriptional control located upstream of the initiation site. The promoter sequence is rich in G+C features, lacks a TATA box and contains potential binding sites for Sp-1 transcriptional factors (Inoue *et al* 1997). Several variables have been reported to influence *PRNP* expression under *in vitro* conditions including nerve growth factor (NGF), rate of prion infection and epigenetic changes (Bueler *et al* 1993, Martins *et al* 2002).

[1] NCJDSU, National Creuztfeldt-Jackob Disease Surveillance Unit. 2009. The University of Edinburgh. http://www.cjd.ed.ac.uk/

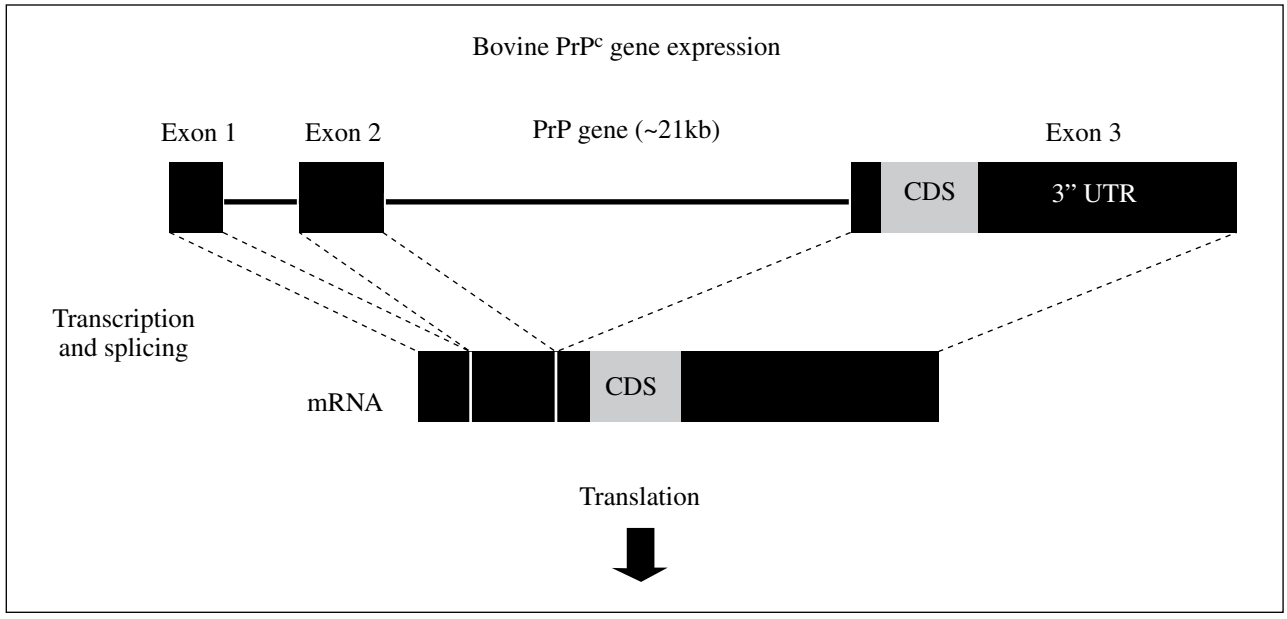

Figure 1. Structure of the *PRNP* gene and mRNA. The *PRNP* gene size is approximate 21 kb. After transcription and splicing, the mRNA molecule is formed by exons 1, 2 and 3. Exon 3 carries the coding sequence that encodes the PrP^C protein after translation.

Estructura del gen *PRNP* y su mRNA. El gen *PRNP* posee un tamaño aproximado de 21 kb. Después de la transcripción y empalme la molécula de mRNA queda conformada por los exones 1, 2 y 3. El exon 3 posee la secuencia que codifica al PrP^C durante la traducción de la proteína.

PrP^C FUNCTION

Despite intense investigation during recent years, the function of PrP^C remains enigmatic. Some studies have suggested a cellular protective role of PrP^C against oxidative stress. Experiments have showed that neurons from *PRNP* knockout mice and cultured *in vitro* displayed higher susceptibility to oxidative agents such as hydrogen peroxide, xanthine oxidase and copper ions compared to wild-type neurons (Brown *et al* 2002). Moreover, brain tissue collected from *PRNP* knockout mice exhibited biochemical changes including increased levels of protein carbonyls and lipid peroxidation products, which are indicative of oxidative damage (Wong *et al* 2001). An extensive body of evidence has suggested the binding of Cu^{+2} ions to the PrP^C octapeptide repeat region. Copper (Cu^{+2}) is an essential element that participates as an enzymatic cofactor in the biochemical pathways of all aerobic organisms. However, Cu^{+2} can also catalyze the formation of reactive oxygen species such as the hydroxyl radical (Martins *et al* 2001). The binding of Cu^{+2} to PrP^C may limit the capacity to catalyze the formation of such toxic oxidative radicals (Martin *et al* 2001, Vassallo and Herms 2003). Alternatively, PrP^C may modulate the activity of the Cu/Zn superoxide dismutase (Cu/Zn SOD) enzyme that showed cellular protective function against oxidative stress (Wechslberger *et al* 2002, Brown *et al* 2002).

Several lines of evidence have proposed a cytoprotective role of PrP^C against internal or environmental stresses that initiate apoptosis. This anti-apoptotic potential is primarily based on the capacity of PrP^C to inhibit the action of the pro-apoptotic protein Bax (Bounhar *et al* 2001). Alternatively, PrP^C may act upstream of Bax, affecting the activity of BH3, Bcl-2 or Bcl-X_L, or downstream, suppressing the effects of Bax in the release of cytochrome c or activation of Apaf-1 and caspases (Roucou *et al* 2005, Westergard *et al* 2007). Other studies have reported a close similarity between the homologous domain of the anti-apoptotic protein Bcl-2 and the PrP^C octapeptide region. This analogy in the protein structure may allow PrP^C to mimic Bcl-2 function and induce cell survival (Roucou *et al* 2005, Westergard *et al* 2007).

In addition to the cytoprotective role, PrP^C has been also implicated as a cell proliferation and differentiation factor. Recently it was reported that PrP^C-null mice exhibited an impaired capacity of self-renewal of hematopoietic stem cell populations after serial transplantation in the bone marrow (Zhang *et al* 2006). The potential mitogenic capacity has also been supported by studies showing a decrease in T lymphocyte proliferation in mice devoid of PrP^C (Bainbridge and Walker 2005). The role of PrP^C in differentiation was suggested by high levels of expression in cells that ceased proliferation and became differentiated into neurons during early stages of mice embryogenesis (Tremblay *et al* 2007). Recently, it has been reported that PrP^C displayed a positive effect in the proliferation of neural precursor cells and showed a positive correlation with neuronal differentiation (Steele *et al* 2006, Peralta *et al* 2011).

The capacity of PrP^C to bind to several different molecules has opened the idea that this protein may exert its function in association with a ligand. The location of PrP^C in the extracytoplasmic face of the lipid bilayer restricts the interaction to transmembrane and secreted proteins. Transmembrane variants of PrP^C could potentially interact with cytoplasmic partners; however, these forms are normally present in low amounts in the absence of predisposing mutations in the PrP^C molecule (Stewart and Harris 2001). The membrane association and the interaction with ligands suggest the hypothesis that PrP^C may activate transmembrane signaling processes associated to neuronal survival, differentiation and neurite outgrowth. The interaction between PrP^C and the stress inducible protein (STI-1) showed high affinity and specificity resulting in neuroprotective functions through the mediators of the cAMP dependent protein kinase (AMPc/PKA) pathway (Zanata *et al* 2002). Additionally, interaction with STI-1 induced neuritogenesis through the MAPK pathway as a parallel effect to neuroprotection (Lopes *et al* 2005). Neuronal growth has also been observed during PrP^C interaction with the neuron cell adhesion protein N-CAM after its recruitment from lipid rafts and the activation of Fyn kinase (Santuccione *et al* 2005). Treatment of cultured neurons with recombinant PrP^C enhances neurite outgrowth and neuronal survival, concomitant with activation of several kinases, including fyn, PKC, PKA, PI-3 kinase/Akt and ERK (Kanaani *et al* 2005, Santuccione *et al* 2005). In addition, studies have showed that PrP^C binds the receptor of laminin in neural cells resulting in dendritic extension, neuronal migration, axonomic regeneration and suppression of cell death induced by kainic acid injection (Martins *et al* 2001).

PRION HYPOTHESIS

The prion hypothesis or protein-only hypothesis postulates that the agent responsible for prion propagation is originated by autocatalytic conversion of PrP^C into the pathogenic isoform (Griffith 1967). Conversion into PrP^{Sc} involves a drastic alteration in the protein configuration as well as in its biochemical properties. Crystallography studies indicate that in normal state 47% of the PrP^C structure is composed of α-helix and only 3% β-sheet secondary configuration. In the conversion process, the β-sheet configuration is increased to 43-45 % and the α-helix structure is reduced to 17-30% (Pan *et al* 1993, DeMarco and Dagget 2005) (figure 2). Therefore, the newly formed PrP^{Sc} structure is highly planar and stable showing strong resistance to temperature, pH, disinfectants and enzymatic degradation (Taylor 2000).

Additional supporting evidence of the prion hypothesis has been originated from studies that reported resistance to prion infection in mice lacking the *PRNP* gene (Bueler *et al* 1993). These knockout models not only evidenced the requirement of a host-encoded PrP^C protein for the infection process but also allowed a better understanding of the pathogenesis of TSEs. However, the most compelling evidence to probe this theory is yet to be reported. Some researchers claimed that a confirmatory experiment will consist in the *in vitro* conversion of PrP^C molecules into a pathogenic isoform with the capacity to induce TSE infection (Chesebro 1998). Mutations induced to recombinant PrP^C have resulted in destabilization of the protein configuration and formation of a PrP^{Sc}-like molecule; however, this mutated agent was unable to induce prion disease (Chiesa *et al* 1998, Bocharova *et al* 2005). It is possible that additional factors including a transitional form of PrP and host-derived proteins or non-protein compounds (chaperones, glycosaminoglycans or short nucleic acids) are required to sustain *in vitro* generation of PrP^{Sc} (Castilla *et al* 2005, Aguzzi *et al* 2007). Hamster PrP^C was only converted to PrP^{Sc} when cell lysate was added to the reaction (Deleault *et al* 2005). Moreover, mice co-expressing both human and mice PrP were resistant to prion replication as consequence of the interaction of mice PrP^C with an additional factor (termed protein X) that inhibited human PrP^C conversion (Telling *et al* 1995). Recent studies reported the *in vitro* generation of PrP^{Sc} molecules using a protein misfolding cyclic amplification technique (PMCA) that allows the repetitive amplification of the misfolding event (Castilla *et al* 2005). Although, the newly formed PrP^{Sc} generated by this technique was able to infect wild-type Syrian hamsters, the use of crude brain homogenates to

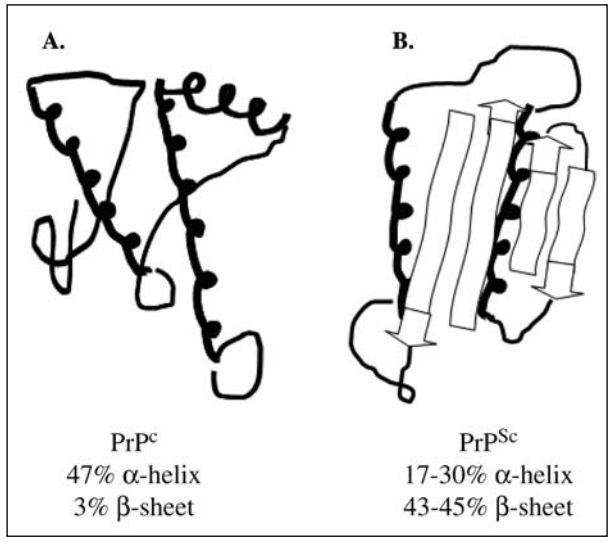

Figure 2. Structure of PrP^C and PrP^{Sc} isoforms. Both isoforms have important differences in secondary protein configuration. PrP^C is structured by a high α-helicoidal (47%) and low β-sheet configuration (3%). In contrast, PrP^{Sc} is characterized by high β-sheet (43-45%) and low α-helicoidal (17-30%) proportion.

Estructura de las isoformas PrP^C y PrP^{Sc}. Ambas isoformas poseen importantes diferencias en su configuración secundaria. La PrP^C está constituida por un alto porcentaje de estructura α-helicoidal (47%) y bajo porcentaje β-planar (3%). En contraste, el PrP^{Sc} es caracterizado con una alta proporción de estructura β-planar (43-45%) y un bajo contenido α-helicoidal (17-30%).

amplificate these molecules may have also resulted in the addition of different components responsible for the infection. Further simplification of PMCA has been reported by substituting shaking for sonication as described for the quaking-induced conversion (QuIC) reactions (Atarashi *et al* 2008). The QuIC assay can detect within one day less than one lethal intracerebral dose (sub-femtogram amount) of PrP^{Sc} in hamster brain homogenates.

Two distinct models have been proposed to explain the autocatalytic conversion of PrP^{C}, a process not mediated by nucleic acids that challenge the central dogma of molecular biology. The template-assisted model postulates a thermodynamically stable conversion between both PrP isoforms (figure 3). PrP^{C} conversion is induced by PrP^{Sc} and is mediated by an intermediate and heterodimeric unit before the formation of a homodimeric PrP^{Sc}. The process is catalyzed by a yet unidentified protein X that has chaperone-like properties and facilitates aggregation of both isoforms (Cohen and Prusiner 1998). Protein X promotes PrP^{C} conversion by binding a discontinuous epitope in the globular C terminal region of the protein (Kaneko *et al* 1997). Newly formed PrP^{Sc} can eventually agglutinate and precipitate, forming amyloid precursors detected in some TSEs (Prusiner 1990, Cohen 1999). A second model termed nucleation-polymerization proposes a similar thermodynamic equilibrium between both isoforms (figure 3). However, after PrP^{C} conversion, the model describes a highly unstable and transient PrP^{Sc} molecule that would be stabilized only by forming ordered aggregates. The stabilized oligomers act as nuclei to recruit monomeric PrP^{Sc} in a process that displaces the thermodynamic equilibrium and accelerates PrP^{Sc} formation (Caughey 2001, Caughey and Lansburry 2003).

Despite a bulk of evidence in support of the prion hypothesis, alternative models suggesting the participation of viral particles, virinos and small RNAs have also been proposed. Co-sedimentation of retroviral RNA with PrP^{Sc}, and purification of short RNA fragments from infectious fractions suggest the participation of nucleic acids as part of the infectious particle (Akowitz *et al* 1994). The virino model describes the TSE agent as a proteinaceous structure containing nucleic acids with a virus-like conformation (Chesebro 1998). The finding that prions have a variety of strains that correlates with a species-specific symptomology and histopathology in TSEs has also been used as evidence to support the virino model (Chesebro 1998). However, the strain phenomenon can be explained by the variation in PrP^{Sc} protein secondary structure and

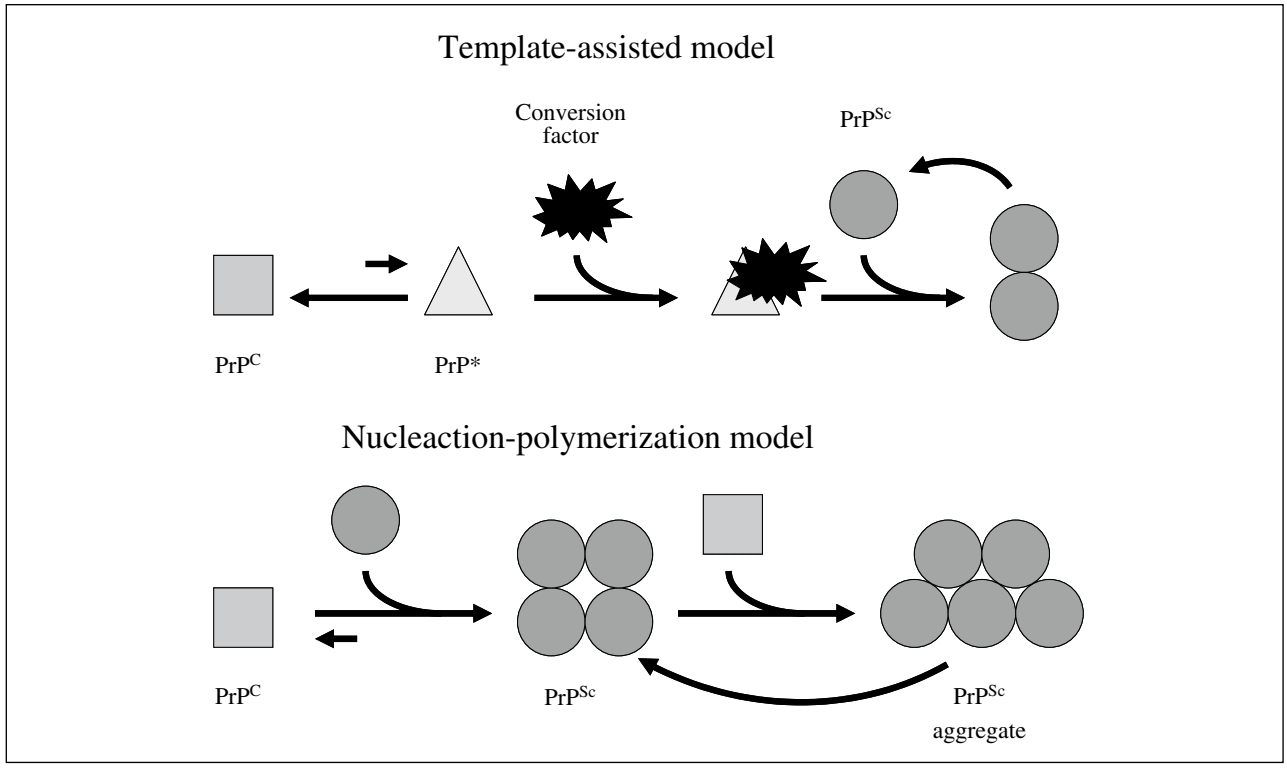

Figure 3. Models for conversion between PrP^{C} and PrP^{Sc}. The template assisted model includes the participation of a conversion factor that mediates PrP transformation. The nucleation-polymerization model describes a PrP^{Sc} heterodimer intermediate complex that induces aggregation and conversion of the molecule. Both models propose a thermodynamic equilibrium for PrP^{C}-PrP^{Sc} conversion (Soto 2006).

Modelos de conversión entre PrP^{C} y PrP^{Sc}. El modelo de patrón asistido incluye la participación de un factor de conversión que media la transformación del PrP. El modelo de nucleación-polimeración describe un heterodimero intermediario del PrP^{Sc} que induce agregación y conversión de la molécula. Ambos modelos proponen un equilibrio termodinámico para la conversión PrP^{C}-PrP^{Sc} (Soto 2006).

not necessarily by the existence of viral strains containing nucleic acids (Prusiner 1998).

PrP^C ROLE IN TSE PATHOGENESIS

The pathogenesis of TSEs including the mechanism of neuronal degeneration has not been completely elucidated. It is now becoming clear, however, that PrP^{Sc} can kill neurons by virtue of its ability to perturb the normal and physiological activities of PrP^C (Westergard *et al* 2007). The presence of PrP^{Sc} alone in neuronal cells may result in toxic effects by several mechanisms including blocking axonal transport, interfering with synaptic function, or triggering apoptotic pathways (Westergard *et al* 2007). Alternatively, the potential association between PrP^{Sc} and PrP^C during the pathogenic process may result in the loss of PrP^C anti-apoptotic activity, resulting in neuronal death. Evidence that argues against this theory is based on the small phenotypic effect and lack of any features of TSEs observed after ablation of PrP^C either prenatally or postnatally (Bueler *et al* 1992, Mallucci *et al* 2002). However, it is possible that neurodegeneration may be consequence of both loss and gain in function, by loss of the cytoprotective activity of PrP^C that may become essential in the disease state due to cellular or organism stress.

Another hypothesis for the TSE-pathogenic effect postulates the alteration or subversion of the normal PrP^C neuroprotective function. PrP^C may act as a membrane-anchored signal transduction that transmits the PrP^{Sc} toxic effect (Chesebro *et al* 2005). Neurons obtained from PrP^C knockout mice and cultured *in vitro* were resistant to apoptosis induced by exposure to the synthetic peptide PrP 106-126 (Brown *et al* 1994). This phenomenon may be the consequence of a PrP^{Sc}-induced aggregation of cell surface PrP^C that generates a neurotoxic rather than a neuroprotective signal. Cross-linking of PrP^C using anti-PrP antibodies resulted in apoptosis of neurons in vivo (Solforosi *et al* 2004). Alternatively, amino acid sequences (PrPΔ 105-125) in the PrP^C structure have been reported to have receptor activity with cytoprotective functions. These receptor sequences may be blocked by PrP^{Sc} resulting in the delivery of a neurotoxic signal (Li *et al* 2007).

BOVINE SPONGIFORM ENCEPHALOPATHY

EPIDEMIOLOGY

Bovine spongiform encephalopathy (BSE) was reported for the first time in the UK in 1986 (Wells *et al* 1987). The disease achieved epidemic proportions during the 1990s with more than 182,000 cases worldwide recorded between November of that year and December of 2010. BSE has been detected in 24 countries showing a decreasing trend in the number of cases since 2003 until 2010 (2167 and 37 cases, respectively) (OIE 2011).

The origin of BSE has not been clarified but several theories have been formulated. One of these theories postulates the inter-species transmission from scrapie-infected sheep to cattle. Thus, cows became infected after consumption of protein concentrate made from carcasses of sheep contaminated with scrapie. Scrapie has affected sheep for 200 years, is endemic in the UK and is present in a number of countries worldwide (Schwartz 2003). Although contamination of feed with infected ovine carcasses is possible; experimental transmission of scrapie agent to the bovine has proved difficult, making this hypothesis improbable (Cutlip *et al* 1994). A second theory involves the spontaneous destabilization and conversion of PrP^C into PrP^{Sc}. Spontaneous or atypical BSE cases have been reported recently, and animals affected by this disease may have served as a source of protein supplements for cattle feed thus spreading the disease in this manner (Capiobianco *et al* 2007). However, one of the most controversial theories to explain the contamination of animal concentrate is the human origin of BSE. Funeral rituals in areas of India involve the cremation of cadavers and the disposal of human remains to rivers. Some of these remains may have been collected by "bonepickers" and used to elaborate bone meal that eventually might have been contaminated with human TSE. By this process, contaminated bone and meat meal exported from the India to Europe and used for animal consumption may have served as a source of BSE transmission (Colchester and Colchester 2005).

CAUSAL AGENT

Detection of PrP^{Sc} is highly correlated with the pathology and diagnosis of BSE. However, the sole presence of PrP^{Sc} as the pathogenic agent of animal TSEs has been debated (Jeffrey and Gonzalez 2007). Mice infected with scrapie agent developed lesions in areas of the brain that showed low levels of PrP^{Sc} (Parchi and Gambetti 1995). Moreover, no brain damage was reported in transgenic mice over-expressing PrP^{Sc} (Chiesa and Harris 2001). These reports suggest that cellular damage is a consequence not exclusively of PrP^{Sc} but also require the participation of additional cofactors including a transitional form of PrP and host-derived proteins or non-protein compounds (glycosaminoglycans or short nucleic acids) (Aguzzi *et al* 2007). In this scenario, PrP^C may play an important role as a mediator in PrP^{Sc} pathogenesis. Mice infected with PrP^{Sc} in which *PRNP* gene was knocked out from the beginning or during the infectious process showed that PrP^C expression is required for cellular damage (Brandner *et al* 1996, Mallucci *et al* 2003).

PATHOGENESIS

There are several origins of prion disease pathogenesis that remain to be understood. It is believed that the BSE agent is passed under natural conditions from one animal to

the other through oral ingestion. Indeed, the transmission of the PrP^{Sc} agent by the oral pathway has great relevance in some TSEs (e.g. variant Creutzfeldt-Jakob disease, Kuru and BSE). This is not the case in spontaneous forms of this disease in which genetic predisposition seems to be the causative factor (e.g. spontaneous Creutzfeldt-Jakob). Although oral ingestion of the agent is the most commonly known form of contamination, other ways of infection cannot be overlooked, such as injection of contaminated products, skin injuries, blood transfusion or iatrogenic ways (Mabbott and MacPherson 2006, Houston *et al* 2008). The transport of PrP^{Sc} through the intestinal mucosa is mediated by microfold cells (M cells) located within the epithelium villus and follicle-associated epithelium (FAE) of the Peyer's patches, through the process of transcytosis (Heppner *et al* 2001). Under physiological conditions, M cells sample contents of the intestinal lumen and present these antigens to the host immune system for immunomodulation. Some pathogenic microorganisms and potentially PrP^{Sc} can exploit M cells transcytosis to gain entry into mucosal tissues (Neutra *et al* 1996). The transport of TSE agents across the intestinal epithelium; however, might not be entirely mediated by M-cell-transcytosis. PrP^{Sc}-proteon complexes originated from CJD brain homogenate can be endocytosed by intestinal epithelial cells (Caco-2 cells) and transcytosed in vesicular structures by a ferritin-dependent mechanism (Mishra *et al* 2004).

After transport through the intestinal mucosa, PrP^{Sc} particles are captured by several types of cells including macrophages, lymphocytes and dendritic cells (DCs) and presented to follicular dendritic cells (FDCs) in the lamina propia. The precise involvement of macrophages in TSE pathogenesis is uncertain but these cells may mediate the transport and also the impairment of PrP^{Sc} accumulation. A recent *in vitro* study showed that macrophage depletion resulted in an earlier increase in PrP^{Sc} accumulation in the lymphoid tissue (Maignien *et al* 2005). Lymphocytes are also situated intraepithelial, but are unlikely to be involved in transporting PrP^{Sc} as they do not acquire measurable levels of this agent following intra-intestinal exposure (Huang *et al* 2002). DCs are a distinct lineage from stromal derived FDCs that sample antigens in the periphery and deliver them to lymphoid tissues to initiate an immune response (Shortman and Liu 2002). The location of DCs beneath intraepithelial M cells and their capacity to acquire intestinal antigens by inserting their dendrites between tight junctions (independently from M cells) make these cells good candidates to transport PrP^{Sc} to lymphoid tissues (Beekes and McBride 2000).

Analysis of the distribution of the PrP^{Sc} within the nervous system of orally inoculated rodents and livestock indicates that the agent subsequently spreads from the gut-associated lymphoid tissue (GALT) to the CNS through the enteric nervous system in a process known as neuroinvasion (Beekes and McBride 2000, Hoffmann *et al* 2007, van Keulen *et al* 2008). The enteric nervous system is an important component of the autonomic nervous system and regulates intestinal motility and secretions through stimuli from sympathetic and parasympathetic nerves. PrP^{Sc} reaches the CNS by spreading in a retrograde direction along efferent fibers of both sympathetic and parasympathetic nerves until they contact the spinal cord. It is not understood how PrP^{Sc} initially spread from the FDCs to the peripheral nervous system. This process may be mediated by mobile intermediate cells or exosomes derived from FDCs, DCs or macrophages that transport PrP^{Sc} particles from FDCs to the peripheral nervous system (Mabbott and MacPherson 2006). The haematogenous route may represent a parallel or alternative pathway of neuroinvasion to ascending infection via the autonomic nervous system. Despite the protective function of the blood-brain barrier, the circumventricular organs are more permissive than other brain areas to the passage of large molecules and may act as portals of entry of infection (Siso *et al* 2010).

HISTOPATHOLOGICAL CHANGES

In general, histopathological changes observed in TSEs are associated with spongiform degeneration, vacuolization, astrogliosis and PrP^{Sc} deposition (Budka 1995, McDonald *et al* 1996). However, astrogliosis and neuronal loss are not evident in BSE compared to other TSEs. The presence of amyloid plaques are rare in classic BSE and are mostly found in the thalamus. In contrast, atypical BSE or Bovine Amyloidotic Spongiform Encephalopathy (BASE) has been characterized by the presence of large plaques mainly in the white matter. The molecular signature of BASE also differs from BSE and resembles CJD (Casalone *et al* 2004). Several theories have been proposed to explain BASE presentation including spontaneous occurrence of TSE in animals as in sporadic CJD. Classic BSE is also characterized by spongiosis and vacuolization of the neuropil and neuronal bodies through simple or multiple vacuoles in the neuronal perikarya (Wells *et al* 1989). Neural tissues most consistently and severely affected are the solitary tract nucleus, the spinal tract nucleus of the trigeminal nerve, and the central gray matter of the midbrain (Wells *et al* 1989). Neuropil vacuolization of the target nuclei is considered to be pathognomic in BSE. Intraneuronal vacuolation is also observed in BSE, but this feature alone in the absence of neuropil vacuolation is not confirmatory. Vacuolated neurons particularly in certain locations such as the red nucleus may be an incidental finding in cattle (Gavier-Widen *et al* 2001). Immunohistochemical analysis consistently reveals PrP^{Sc} accumulation in the brain, with distribution similar to but often more widespread than neuropil vacuolization (Wells and Wilesmith 1995). Patterns of PrP^{Sc} deposition in the brain include intraneuronal, perineuronal, linear, fine punctuate and coarse particulate (Casalone *et al* 2006). PrP^{Sc} cannot readily be detected in tissues outside bovine CNS; however, limited

involvement of the Peyer's patches has been documented in experimentally induced and naturally acquired cases of BSE (Terry *et al* 2003).

DIAGNOSIS

All cattle BSE diagnostic methods currently available require post-mortem confirmation. Despite characteristic clinical signs, diagnosis of BSE cannot rely solely on the clinical course and requires histopathological analysis. Initial histopathological diagnosis is based on vacuolar changes in the brain, observation of florid plaques, astrogliosis and neuronal loss. However, presentation of cases with no evident or ambiguous histopathological changes requires confirmation through more specific and sensitive methods such as immunohistochemistry, western blot and/or ELISA (Gavier-Widen *et al* 2005, OIE 2004). Given the pivotal role played by PrP^{Sc} in TSE pathogenesis, diagnostic methods rely on PrP^{Sc} detection by specific antibodies and partial proteinase K digestion that allows differentiation between both PrP isoforms. In recent years, some antibodies have claimed to differentiate between PrP^{C} and PrP^{Sc}; however, none of these molecules have proved suitable for direct identification of PrP^{Sc} (Korth *et al* 1997, Curin *et al* 2004).

Consistent early accumulation of PrP^{Sc} and vacuolar lesions in the medulla oblongata at the level of the obex (brainstem) make this area of the brain an optimal site for the post-mortem diagnosis of BSE (Schaller *et al* 1999). An appropriate sample for BSE diagnosis should include the solitary and trigeminal tract nuclei (Jeffrey and Gonzales 2004). The brainstem sample should be promptly refrigerated (at 4 °C not frozen) or fixed in 10% formaldehyde until shipment to the diagnostic laboratory to avoid post-mortem decomposition (Gavier-Widen *et al* 2005, USDA 2005).

Several diagnostic methods based on immunological techniques have been validated and officially accepted for BSE. Currently, the BioRad diagnostic immunoassay is used as a rapid test for BSE detection. Advantages of this method include easy manipulation, fast results (24 h), high sensitivity, and detection of PrP^{Sc} infection during preclinical stages (Soto 2006). However, presence of false positive results recommends the use of confirmatory techniques (USDA 2005). High specificity, visualization of the spongiform lesions and PrP^{Sc}-specific immunostaining make the immunohistochemistry a primary choice for confirmatory test for BSE diagnosis. Mounted tissue sections are exposed to proteinase K digestion and epitope unmasking through immersion in citric buffer. PrP^{Sc} is detected by incubation with anti-PrP antibodies followed by secondary antibodies associated to horseperoxidase staining or fluorescence dyes (OIE 2004). Samples with moderate levels of decomposition may not be suitable for the immunohistochemistry method but may be used for western blot analysis. This method has similar specificity compared to immunohistochemistry but does not allow histological analysis. Currently, this technique can be performed in a short period of time with high sensitivity. Tissue sample lysates are treated with proteinase K for PrP^{C} digestion. Total protein contained in samples are separated by electrophoresis in 12% SDS polyacrylamide gels and blotted into nitrocellulose or polyvinylidene fluoride (PVDF) membranes. PrP^{Sc} is specifically detected by incubation with anti-PrP antibodies followed by secondary antibodies.

The requirement of a pre-mortem diagnostic method is desirable considering the inability to apply conventional methods for nucleic acids or antiserum detection such as PCR or ELISA. During the last years, substantial efforts have been applied in the development of PrP^{Sc}-specific antibodies for the potential diagnostic of infectivity in fluids and tissues. Despite the significant differences in secondary structure between both PrP isoforms, the development of such antibodies is still incomplete (Kascsak *et al* 1997, Curin *et al* 2004). PrP^{Sc} has been detected in the blood of infected animals, which represents a potential strategy for early diagnosis of TSEs (Castilla *et al* 2005). However, levels of PrP^{Sc} in blood are very low making even highly sensitive techniques such as immunocapillary electrophoresis unable to detect the agent (Schmerr and Jenny 1998). One important step in the development of such a diagnostic tool was the recently reported development of a protein misfolding cyclic amplification technique (PMCA). This method was able to simulate PrP^{Sc} replication in a test tube and increase the detection threshold of this protein by 10 million times (Castilla *et al* 2005, Thorne and Terry 2008). This new technology enables an efficient, specific and rapid detection of prions offering great promise for developing a noninvasive early diagnosis of TSEs.

CONCLUSIONS

The establishment of the controversial prion hypothesis opened a new perspective in protein biology that involved the participation of these molecules in diseases as pathogenic and infectious agents. Not only TSEs, but other neurodegenerative diseases such as Alzheimer's have shown to be associated with the misfolding and deposition of host-encoded proteins with severe neuropathology. Currently, there are no ways to cure, treat or immunize against these diseases, and the consequences for public health and economic costs have proven to be tremendous. However, important advances during the last years in the evaluation of prion biology have allowed a better understanding of the pathogenesis of these diseases and have opened new opportunities for treatment.

SUMMARY

The complex nature of prions has intrigued the scientific community during the last 70 years. Since the first indication of scrapie infectivity

and the experimental transmission of the scrapie agent in 1937, prions and their associated transmissible spongiform encephalopathies (TSEs) have been under constant investigation. TSEs are neurodegenerative and fatal diseases with no early diagnosis, treatment or cure. Despite their diverse presentations, all TSEs stem from the infectious, spontaneous or hereditary conversion of the host-encoded cellular prion protein PrP^{C} into the pathogenic isoform PrP^{Sc}. Based on the prion hypothesis, PrP^{C} has the autocatalytic or induced capacity to change its secondary configuration from a mainly α-helix structure into predominant β-sheet configuration. Another enigmatic aspect of the prion biology is the potential physiological function of PrP^{C}, a protein that is widely distributed in mammalian tissues and intensely expressed in the nervous system. PrP^{C} has been associated to several biological roles including cellular adhesion, protection and differentiation. The unpredictable properties of the PrP^{Sc} and the complex presentation of TSEs have opened many questions yet to be answered. The potential zoonotic transmission of the bovine spongiform encephalopathy (BSE) has generated intense concern in the international community over animal product biosecurity. During the last years, research in prion biology has mainly focused on determination of the pathogenesis of TSEs and the development of diagnostic and therapeutic methods. However, further research in prion biology is required in order to understand the complex nature of TSEs and how these diseases can be controlled.

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