Host and transmissible spongiform encephalopathy agent strain control glycosylation of PrP

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PrP is a host-encoded glycoprotein involved in the pathogenesis of transmissible spongiform encephalopathies (TSEs) or 'prion' diseases. The normal form of the protein (PrPC) is heavily but incompletely glycosylated; it shows structural diversity in three neuroanatomically distinct regions of the brain. No effect of TSE infection on PrPC glycosylation has been detected. TSE-specific forms of PrP (PrPSc) vary in their degree of glycosylation according to strain of TSE infectious agent. PrPSc also varies independently in the amount and pattern of glycosylation according to brain region. This diversity shows that the glycosylation of PrP is under both host- and TSE agent-specified control, probably within the biosynthetic pathway for protein N-glycosylation. These findings challenge assumptions that PrPSc is formed from the normal, mature form of PrPC but are compatible with a model in which the glycosylation phenotype of PrPSc is under the control of both host cellular factors and TSE agent-specified information.

Introduction

The group of diseases known as transmissible spongiform encephalopathies (TSEs) cause a progressive degeneration of the central nervous system. There is much controversy about the nature of their causal agent. In the 'protein-only' or 'prion' hypothesis, a hypothetical form of PrP (called PrP* (Aguzzi & Weissmann, 1997) or a subfraction of PrPSc (Prusiner, 1997)) comprises the entire infective particle. 'Infectious PrP' co-purifies with other abnormal forms of PrP. The virino hypothesis proposes that PrP protein is one component of the infective particle and that an agent-specific informational molecule (expected to be nucleic acid) encodes the genetic information required to determine TSE strain properties of incubation period, distribution of brain lesions and PrPSc glycoform profile (Farquhar et al., 1998). Whatever the role of PrP in the structure of the agent, it is now clear that PrP is the product of the Sinc gene (Moore et al., 1998), which controls agent replication (Dickinson & Meikle, 1971).

Two PrP forms are operationally defined by their biochemical properties. The PrPC fraction is protease-sensitive, soluble in detergents (Meyer et al., 1986) and released from the surface of tissue culture cells by phosphoinositol phospholipase C (PIPLC) (Stahl et al., 1990a). PrPC is considered to be the normal form of the protein, present in both uninfected and infected tissues. The PrPSc fraction, which is found only in TSE-affected tissues, is partially protease-resistant (Meyer et al., 1996) and is not released by PIPLC (Stahl et al., 1990a). It has been proposed that PrPSc (including the hypothetical infectious form in the protein-only hypothesis) is formed from the mature form of the normal protein; i.e. PrPC → PrPSc (Prusiner, 1991).

The primary structure of the mature PrP protein comprises approximately 210 amino acids. It has two N-glycosylation sites (Oesch et al., 1985) and a C-terminal glycosylphosphoinositol (GPI) anchor (Stahl et al., 1990b). Most PrPC is glycosylated at both sites, while PrPSc varies in the amount of glycosylation (Kascak et al., 1985; Parchi et al., 1995; Collinge et al., 1996; Somerville et al., 1997b). Much of the structural diversity of PrP arises from the incomplete addition of carbohydrate moieties (Somerville & Ritchie, 1990) at the N-glycan sites (Oesch et al., 1985), which have a multiplicity of core carbohydrate structures (Endo et al., 1989), and differential addition of neuraminic acid (Endo et al., 1989). Most analyses have been performed on PrPSc extracts, but there is no evidence of structural differences at the molecular level between PrPC and PrPSc (Stahl et al., 1993), other than changes caused by partial protease digestion of PrPSc (Hope et al., 1986) and the degree of N-glycosylation.

In this paper, biological parameters that affect differences in PrP glycoform patterns are investigated to determine whether they are host- or agent-derived, by comparing PrPC and PrPSc in three regions of brain. The differences in the degree of glycosylation of PrPSc from brains affected by different TSEs.
strains show that the TSE agent controls PrP<sup>Sc</sup> glycosylation (Kascsak et al., 1985; Somerville et al., 1997b), although the mechanism by which these differences are generated is not known. The diversity of PrP glycosylation is explored further in the present study. If mature, glycosylated PrP<sup>C</sup> is the substrate for conversion of its secondary structure to the PrP<sup>Sc</sup> form, with no other structural alterations, then it would be expected that their glycoform profiles would always be similar. This is not the case. Similarly, if the glycoform profile of PrP<sup>Sc</sup> is dictated solely by the TSE agent, then no differences would be expected in PrP<sup>Sc</sup> from sources where the agent strain is the same but other biological parameters are different. Again this is not the case. The simplest explanation of these findings is that PrP<sup>C</sup> and PrP<sup>Sc</sup> follow biosynthetic pathways that can be regulated independently by both host- and agent-specific factors.

**Methods**

- **Tissues.** VM mice (Sinc<sup>Bg</sup> or Prn<sup>p307</sup>) and SV, C57BL or C57BL mice with the B<sub>g</sub> mutation (all Sinc<sup>s</sup> or Prn<sup>-p307</sup>) were injected intracerebrally with a 1% homogenate from mice infected with the ME7, 22A, 79A or 301V strains of TSE or uninfected control brain. LVG hamsters were injected with the 263K scrapie strain. When clinical signs were apparent, brains were removed, frozen immediately in liquid N<sub>2</sub> and stored at −70 °C. For analysis, brains were either thawed and the whole brain homogenized or a simple dissection was performed to obtain the cortical lobe (cortex), cerebellum and medulla, which were then homogenized individually.

- **Tissue extractions.** For two-dimensional electrophoresis analysis (Fig. 1), tissues from uninfected C57BL mice were dissected and homogenized in 9 vols 0.32 M sucrose, 0.1 vols 100 mM PMSF and N-ethylmaleimide (NEM). Homogenate (0.5 ml) was overlaid on 0.4 ml 1.4 M and 0.85 ml 0.65 M sucrose and centrifuged in a TLS55 rotator at 166000 g for 30 min at 4 °C. The top 1 ml myelin fraction was discarded and the 1 ml membrane fraction was diluted with 1 ml water, 20 µl PMSF, 20 µl NEM, 1 ml Tris–HCl (200 mM, pH 9–10) and 1 ml 4% Sarkosyl and centrifuged in a TL100 rotor at 412000 g for 30 min at 20 °C. Supernatant fluids were precipitated with 2 vols 2% acetic acid in ethanol. To analyse PrP<sup>C</sup> on one-dimensional gels (Fig. 2), uninfected tissues from SV mice were dissected, homogenized in 9 vols 100 mM Tris–HCl, pH 7–4, 0.1 vols 100 mM PMSF and NEM. To analyse both PrP<sup>C</sup> and PrP<sup>Sc</sup> (Fig. 3), control or scrapie-infected brain (mouse strain SV, hamster strain LVG) was homogenized in 9 vols sucrose (0.32 M) and made to 100 mM Tris–HCl, pH 7–4, 1 mM EDTA, 1 mM PMSF, 1 mM NEM and 1% Sarkosyl. One ml aliquots were overlaid on 0.4 ml 20% sucrose, 100 mM Tris–HCl, pH 7–4 and 1 mM EDTA and centrifuged at...
200 000 g for 30 min at 20 °C in a TLS55 rotor. Pellets were resuspended in 100 µl water. An 8 µl aliquot was digested with proteinase K (20 µg/ml) at 37 °C for 30 min.

- **Gel electrophoresis.** Two-dimensional non-equilibrium pH gradient–SDS (NEPHGE–SDS) electrophoresis was performed according to the method of O’Farrell *et al.* (1977) with minor modifications (Somerville *et al.*, 1989). Gels were immunoblotted onto nitrocellulose and developed by using a rabbit polyclonal anti-PrP antibody (1B3; Farquhar *et al.*, 1994) at 1:1000 dilution, visualized with gold-labelled secondary antibody and enhanced with silver. Blots were scanned by reflection and the degree of staining in each area was integrated by using Molecular Dynamics software.

One-dimensional SDS–PAGE was performed according to the method of Neville (1971) and the gels were immunoblotted (Towbin *et al.*, 1979) onto Immobilon-P membrane (Millipore). Blots were developed with ch1659 chicken polyclonal anti-PrP antibody at 1:1000 dilution (Somerville *et al.*, 1997a) (Figs 2–4) or with dH4 anti-PrP monoclonal antibody at 1:50 000 dilution (Korth *et al.*, 1997) (Figs 5–7) and visualized by chemiluminescence (Boehringer). The films were scanned by transmission and data were analysed by using a Molecular Dynamics densitometer and Microsoft Excel. To obtain the values for aglycosyl (U), monoglycosyl (L) and diglycosyl (H) forms of PrP, each gel lane was scanned, a densitometric trace of the lane was obtained and divided into H, L and U by inspection and the area of each of these parts of the scan was calculated. Each comparison (except Fig. 1) shows results from a single gel on which groups of four separate tissue extracts were compared. Student’s t-test was performed on the means of % H, % L and % U for each sample group.

**Results**

**Diversity of PrPC structure**

The source of heterogeneity of PrP glycosylation was explored in an initial experimental series by examining the glycoform patterns of PrPC in extracts from various parts of uninfected mouse brain. PrPC from whole brain, cortex, cerebellum and medulla was compared on two-dimensional NEPHGE–SDS (2D) gels after immunoblotting. The three major bands, representing aglycosyl (U), monoglycosyl (L) and diglycosyl (H) forms of PrP, were identified by reference to previous analyses that had correlated the degree of glycosylation to the migration of PrPSc on NEPHGE–SDS gels (shown in the high-contrast image of whole brain; Fig. 1b) (Somerville & Ritchie, 1990). Most PrPC was completely glycosylated but there were some differences in the degree of glycosylation (Fig. 1a, c). Densitometric analysis showed that PrPC from medulla had a significantly higher degree of glycosylation than that from cortex or cerebellum (Fig. 1c). To confirm this observation, one-dimensional SDS–PAGE (1D) of PrPC was performed. This method did not separate the three glycoforms of PrPC sufficiently to allow their individual quantification; nevertheless, consistent differences in banding could be observed (Fig. 2). Small differences could also be seen within each band on both 1D and 2D gels, e.g. within the H and L bands of PrPC from cortex, cerebellum and medulla on the 1D gels [Fig. 2; see also the inserts of the L and U bands from cerebellum and medulla in Fig. 1(a)]. It is concluded that cellular factors differentially control PrPC glycosylation.
To determine whether PrP^Sc was altered by infection, the PrP^C fractions from brains of scrapie-infected animals were compared with PrP^C from control brain. Models with a low glycoform ratio (79A) and a high glycoform ratio (263K) of PrP^Sc glycosylation were selected (Somerville et al., 1997b). To enable an examination of both PrP^C and PrP^Sc from the same brain, tissue extracts were solubilized in Sarkosyl and subjected to differential centrifugation. Under the conditions used, PrP^C is sedimented, while PrP^C remains in the supernatant fluid (Meyer et al., 1986). It should be noted that, prior to exogenous proteinase K digestion, the 1D SDS–PAGE pattern includes a mixture of intact PrP and endogenous protease-digested PrP molecules, the M_r of which is about 3000 less (Hope et al., 1988; Somerville et al., 1989). For example, some aglycosyl PrP^Sc (U), which had not been proteinase K-treated (Fig. 3, lane 3) but had presumably been endogenously digested, co-migrated with aglycosyl PrP^Sc after proteinase K treatment (Fig. 3, lane 4). In both cases, the PrP^C fraction from scrapie-infected brain was similar to PrP^C from control brain. By contrast, the PrP^Sc fraction from the same 79A-infected brains was very poorly glycosylated (Fig. 3, lanes 1–4). Similarly, the PrP^C fractions from 263K-infected hamster brains differed little from PrP^C from uninfected animals but, in this case, the PrP^Sc fraction was similar to the PrP^C fraction (Fig. 3, lanes 5–8). This observation suggests that there is no direct relationship between PrP^C and the formation of PrP^Sc and that TSE strain does not affect the glycosylation of PrP^C.

**Diversity of PrP^Sc structure**

A wide diversity in PrP^Sc glycosylation between scrapie strains has been shown previously (Somerville et al., 1997b), showing that TSE strain controls the glycosylation of PrP^Sc. Typical results from three areas of brains infected with four TSE strains are shown in Fig. 4 and for whole brains infected with three TSE strains in Fig. 5. There was relatively weaker binding to the higher glycoforms with the ch1659 chicken polyclonal antibody (Figs 3 and 4) than with the 6H4 monoclonal antibody (Figs 5–7). This is illustrated by the difference in pattern between the ME7 samples in Figs 4 and 6 and is probably due to steric hindrance from carbohydrate moieties near some of the epitopes recognized by the polyclonal antibody. As found previously, there was a large and significant difference in the degree of glycosylation of PrP^Sc from 79A-infected and ME7- or 22A-infected brains. There was only a small difference between PrP^Sc from ME7- and 22A-infected brains (Fig. 5c), significant only for % H between ME7/VM and 22A/VM. There was a small difference, which was not significant, between PrP^Sc glycosylation from ME7-infected SV and VM brains.

To determine whether PrP^Sc glycosylation was under host control, glycoform ratios were determined in cortex, cerebellum and medulla of ME7-infected brains. Significant PrP^Sc heterogeneity was found between brain regions in this model (Fig. 6), e.g. PrP^Sc in the cerebellum of brains infected with the ME7 strain was more highly glycosylated than PrP^Sc from cortex or medulla (Fig. 6c). In brains from VM mice affected with the 301V strain, PrP^Sc from cerebellum was significantly less glycosylated than that from cortex (% H, P < 0.002) or medulla (% H, P < 0.0005) (results not shown). Fig. 4 also illustrates this point where the three brain regions from four TSE strains were surveyed, although this experiment examined single samples and therefore cannot be analysed statistically. It is concluded that host cellular factors as well as the TSE agent factors control PrP^Sc glycosylation.

Variation could also be seen in the distribution within each band, particularly the monoglycosylated (L) band, where a doublet could be observed. For example, in PrP^Sc from 79A- and ME7-infected brain from SV mice there was more staining in the lower part of the band (L1) (Fig. 5a, b; lanes 1, 2), but PrP^Sc from brains of ME7- and 22A-infected VM mice...
Control of PrP glycosylation

Fig. 6. Comparison of PrPSc from ME7-infected SV mouse brain areas. (a) Gels of cortex (Cx, lane 1), cerebellum (Cm, 2) and medulla (Ma, 3). (b) Gel scans averaged for each tissue source (n = 4). (c) Glycoform analysis of differences between regions. Differences were significant for cortex and cerebellum (% H, P < 0.0001; % L, P < 0.00005), cortex and medulla (% H, P < 0.05) and cerebellum and medulla (% H, P < 0.0001; % L, P < 0.0005). Samples were prepared as in Fig. 4.

Fig. 7. Comparison of PrPSc from brains of mice with the Bg mutation with other mouse strains. (a) SDS–PAGE of whole, ME7-infected brains from RIII, SV, C57BL and C57BL mice with the Bg mutation (Bg). (b) Gel scans averaged for each tissue source (n = 4). (c) Glycoform analysis of samples. There were no significant differences between samples. Samples were prepared as in Fig. 4.

contained more of the upper part of the band (L2) (Fig. 6 a, b; lanes 3, 4). There was a predominance of L1 in all three brain regions of ME7-infected SV mice but there was more L2 in medulla than in cerebellum (Fig. 6). Analysis of regions of brains infected with different agent strains suggested that the relative amount of L1 and L2 was affected by both agent strain and sometimes brain region. L1 was predominant in all brain areas from 301V- and 263K-infected brains, while L2 was more prominent in 79A- and ME7-infected areas (Fig. 4), although L1 was more prominent in ME7-infected medulla than cerebellum or cortex (Figs 4 and 6). Hence, for this doublet, the strain of agent, region of brain and PrP genotype of mouse each affected the banding pattern observed. It is possible that the Thr/Val polymorphism at codon 189 in mice, between the two N-glycosylation sites at codons 180 and 196, plays a part.

To test whether the PrPSc glycosylation pattern arose catabolically, PrPSc from ME7-infected C57BL mice carrying the Bg mutation, which causes a lysosomal disorder (Oliver & Essner, 1973), was compared with PrPSc from three other ME7-infected mouse strains (C57BL, SV and RIII). All mouse strains were of the Sin<sup>777</sup> genotype. All four sources were indistinguishable, suggesting that neither the lysosomal deficit nor other genetic differences between these mouse strains had a confounding effect on PrPSc metabolism (Fig. 7).

Discussion

Can TSE strains be identified from their glycoform profiles?

The data show that within individual brains differences in glycosylation exist for PrP<sup>C</sup> (Figs 1 and 2), between PrP<sup>C</sup> and PrP<sup>Sc</sup> (Fig. 3) and for PrP<sup>Sc</sup> (Figs 4 and 6). The glycosylation of PrP<sup>Sc</sup> depends not only on strain of agent (Kacsak <em>et al</em>.., 1985; Somerville <em>et al</em>.., 1997) but also on the region of brain and PrP genotype. Recently, it has been reported that no significant PrP<sup>Sc</sup> glycoform differences were detected in different areas of brains infected with three strains of TSE (Kucziius <em>et al</em>.., 1998). This is surprising in view of the data presented here, where
statistically significant differences were found between the different brain areas. Differences in the pattern of PrP^{Sc} from different parts of the brain have previously been observed on silver-stained, two-dimensional NEPHGE–SDS gels (Somerville & Ritchie, 1989). Differences have also been found between PrP^{Sc} from brains and spleens of mice infected with the 139A strain of scrapie (Rubenstein et al., 1991) and between brains and tonsils from cases of new variant Creutzfeldt–Jakob disease (nvCJD) (Hill et al., 1999).

PrP^{Sc} glycosylation from sporadic CJD cases differs according to PrP genotype and clinicopathological presentation (Parchi et al., 1995), and PrP^{Sc} from sporadic CJD cases segregates from PrP^{Sc} from nvCJD cases (Collinge et al., 1996). Since differences in glycosylation are controlled by both host and agent, they presumably arise as a consequence of the interaction between the infective agent and the host, as a phenotypic property of that interaction. Although there can be wide diversity in glycoform ratios of different TSE strains, some strains have similar ratios in both rodents (Somerville et al., 1997b) and sheep (Hope et al., 1999). The data reported herein from experimental models of TSEs suggest that there can be greater variation in the glycoform profile of PrP^{Sc} from different parts of the same brain than previously reported. Questions therefore arise about the potential use of glycoform analysis for TSE strain typing (Collinge et al., 1996; Hill et al., 1998), at least until the parameters that may affect glycoform ratios that are examined here (the area of human or ruminant brain sampled), and other parameters (e.g. route of infection), have been explored further.

Potential origins of PrP^{Sc} diversity

The origin of the differences in glycosylation of PrP^{Sc} is not known, nor can it be deduced immediately from information on other glycoproteins. A priori, hypothetical sources include partial catabolic digestion of the carbohydrate (i.e. deglycosylation), selection from the PrP^{C} pool or selective control of the biosynthetic pathway.

Deglycosylation hypothesis. Catabolic change might have been detectable if it was occurring slowly in tissues where infectivity replicated early after infection and was followed by a plateau. No change with time was detected in the degree of glycosylation of PrP^{Sc} in spleens from mice infected with ME7 (Farquhar et al., 1994). It might also have been detected as a failure in catabolism in mice with the Bg mutation, but no difference was detected. Deglycosylation of PrP^{C} by digestion of carbohydrate on the mature protein would require specific glycosidases. Direct amino acid sequencing of aglycosyl hamster PrP showed asparagine at the second sequon (Stahl et al., 1993), not aspartate, which would be left as a result of the removal of carbohydrate by mammalian peptide N-glycanase F (Suzuki et al., 1994). The amino acid in the first sequon could not be analysed (Stahl et al., 1993). Since PrP^{C} and PrP^{Sc} glycoform patterns can vary between brain regions, host and agent controls must regulate the degree and pattern of glycosylation. A deglycosylation hypothesis would require differential location- and agent-specified regulation of hypothetical catabolic enzymes. Overall, there is no evidence to support a deglycosylation hypothesis.

Selection hypothesis. PrP^{C} glycoforms in control and scrapie-infected brain are similar, irrespective of the PrP^{Sc} glycoform. Depletion of specific PrP^{C} glycoforms in scrapie-infected brain would have been evidence of their selection from the mature population of PrP^{C}. Moreover, experiments that have attempted to mimic the conversion of PrP from a protease-sensitive to a protease-resistant form in vitro have shown that the conversion occurs whether the incorporated PrP molecules are glycosylated or not (Kocisko et al., 1994). In vivo methods for selecting a subpopulation of mature PrP^{C} to form a PrP^{Sc} fraction would require recruitment from the extant PrP^{C} population, by a mechanism with locational and agent-specific properties. A regulatory mechanism for this process would also have to accommodate the locational differences in both PrP^{C} and PrP^{Sc} glycosylation. Overall there is no evidence to support the suggestion that PrP^{Sc} is selected from the mature PrP^{C} population at the cell surface.

Biosynthetic control hypothesis. Mutation of the PrP N-glycosylation sites alters the PrP biosynthetic pathway in cell culture, resulting in the production of protein with properties similar to PrP^{Sc} (Lehmann & Harris, 1997). Similarly, the intracellular distribution of PrP is altered in the brains of transgenic mice in which exogenous PrP genes have been mutated to prevent glycosylation (DeArmond et al., 1997). Intracellular biosynthetic control of the degree of glycosylation (i.e. of site occupancy) is indicated in this paper by the differences in PrP^{C} between brain regions, and it most readily explains the differences between PrP^{C} and PrP^{Sc} between PrP^{Sc} from different TSE strains and from different parts of the same infected brain. Cellular regulation of the structures of the carbohydrates added (i.e. site processing) is suggested by the differences within the mono- and diglycosylated bands. In scrapie-infected N2A cells, it was shown that the PrP population that becomes PrP^{Sc} behaved initially like PrP^{C} (i.e. it was proteinase K-sensitive and was released by PIPLC) and was located on the cell surface (Caughey & Raymond, 1991). Incorporation of protein into the PrP^{Sc} fraction probably occurred at the cell surface; however, the degree of glycosylation of the PrP^{Sc} fraction was lower than that of the PrP^{C} fraction (Caughey & Raymond, 1991). N-glycosylation of other proteins can be both cell-type-specific and site-specific (Parekh et al., 1989). Overall, these data suggest that more regulatory information is required to control glycosylation than is encoded in the nascent polypeptide chain of PrP. It is concluded that in an infected cell a separate population of PrP molecules, destined to become PrP^{Sc}, is synthesized via a pathway modulated by the infectious agent.
Passage of two natural sources of TSE infection into mice each produced two altered experimental TSE strains that showed discrete biological properties and glycoform patterns (Somerville et al., 1997b). Presumably, changes in the properties of the agent are accompanied by changes in PrP processing: the agent alters the controls it operates on glycosylation in the biosynthetic pathway.

**Role of PrP\(^{Sc}\) in TSE agent structure and pathogenesis**

The prion hypothesis proposes that infection converts the normal form of PrP into an abnormal, infectious form. In some exegeses of the hypothesis (Aguzzi & Weissmann, 1997; Prusiner, 1997), no role for glycoform differences is proposed. However, the existence of different PrP\(^{Sc}\) and PrP\(^{S\text{c}}\) glycoforms implies that PrP\(^{Sc}\) does not originate from in vivo conversion of the mature PrP\(^{C}\) population present on normal cells. Others have suggested that some of the genetic information required by TSE infective agents could be carried by different glycoforms of PrP (Collinge et al., 1996). Diversity of PrP\(^{Sc}\) glycoform profiles in individual brains raises doubts about whether such a novel biological mechanism could encode and transmit genetic information and do so with fidelity.

Whatever the role of the carbohydrate moieties on PrP, it is likely to be similar to one of their roles on other proteins, possibly for recognition of PrP by a lectin. The requirement for PrP recognition may come from targeted movement of infectivity from cell to cell. Some GPI-anchored proteins are released from the cell surface and translocated to adjacent cell surfaces (Kooyman et al., 1995). Although this phenomenon has not been observed for PrP, PrP\(^{C}\) is anchored on the exterior of the plasma membrane and some PrP is released into cell culture medium (Caughey, 1993). In scrapie-infected mouse brain, electron microscopic observations suggest that PrP is released from the extracellular space and then aggregates into fibrils (Jeffrey et al., 1994). Perhaps PrP can translocate to an adjacent cell, identifying the destination cell through the specific recognition properties of PrP carbohydrate moieties; TSE agent strains may modify carbohydrate addition in a strain-specific fashion, constrained by the available host cell mechanisms. Accordingly, the following model is proposed. Normally, a population of PrP molecules is synthesized under differential post-transcriptional control; if a TSE agent modulates the biosynthetic pathway, a new population of PrP is destined to carry the informational molecule predicted by the virino hypothesis (Farquhar et al., 1998) to a recipient cell and also to be deposited as 'PrP\(^{Sc}\)'. In this model, the TSE agent encodes the information required for strain-specific, host–interactive regulation of glycoform diversity. Such a requirement fits well with models proposing an independent informational molecule (expected to be a nucleic acid) that also specifies other strain-specific properties (Somerville, 1991; Bruce et al., 1997; Farquhar et al., 1998).

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