Host and transmissible spongiform encephalopathy agent strain control glycosylation of PrP

Robert A. Somerville

Neuropathogenesis Unit, Institute for Animal Health, West Mains Road, Edinburgh EH9 3JF, UK

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 infective particle and that an agent-specific informational hypothesis proposes that PrP protein is one component of the virino that 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 infective particle and that an agent-specific informational hypothesis proposes that PrP protein is one component of the infectious virino. 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 glycosylphosphatidylinositol (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 TSE

Author for correspondence: Robert A. Somerville.
Fax + 44 131 668 3872. e-mail robert.somerville@bbsrc.ac.uk

0001-5967 © 1999 SGM

Printed in Great Britain
strains show that the TSE agent controls PrP<sub>Sc</sub> glycosylation (Kascak 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<sub>C</sub> is the substrate for conversion of its secondary structure to the PrP<sub>Sc</sub> 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<sub>Sc</sub> is dictated solely by the TSE agent, then no differences would be expected in PrP<sub>Sc</sub> 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<sub>C</sub> and PrP<sub>Sc</sub> follow biosynthetic pathways that can be regulated independently by both host- and agent-specific factors.

**Methods**

- **Tissues.** VM mice (Sin<sup>Btg</sup>, or Prn<sup>p-1</sup>) and SV, C57BL or C57BL mice with the B<sub>g</sub> mutation (all Sin<sup>Btg</sup>, or Prn<sup>p-1</sup>) were injected intracerebrally with a 1% homogenate of brain 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 rotor at 160,000 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 9–0) and 1 ml 4% Sarkosyl and centrifuged in a TL100.3 rotor at 412,000 g for 30 min at 20 °C. Supernatant fluids were precipitated with 2 vols 2% acetic acid in ethanol.

To analyse PrP<sub>C</sub> 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<sub>C</sub> and PrP<sub>Sc</sub> (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 (Somerville et al., 1997a) (Figs 2–4) or with 6H4 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 PrP<sub>C</sub> structure**

The source of heterogeneity of PrP glycosylation was explored in an initial experimental series by examining the glycoform patterns of PrP<sub>C</sub> in extracts from various parts of uninfected mouse brain. PrP<sub>C</sub> 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 PrP<sub>Sc</sub> on NEPHGE–SDS gels (shown in the high-contrast image of whole brain; Fig. 1b) (Somerville & Ritchie, 1990). Most PrP<sub>C</sub> was completely glycosylated but there were some differences in the degree of glycosylation (Fig. 1a, c). Densitometric analysis showed that PrP<sub>C</sub> 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 PrP<sub>C</sub> was performed. This method did not separate the three glycoforms of PrP<sub>C</sub> 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 PrP<sub>C</sub> 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 PrP<sub>C</sub> glycosylation.

**Fig. 2.** 1D SDS–PAGE comparison of PrP<sub>C</sub> from uninfected SV mouse brain. (a) Gels of cerebellum (Cm, lane 1), cortex (Cx, lane 2) and medulla (Ma, lane 3). 35k and 30k indicate the positions of 35 and 30 kDa markers. (b) Gel scans were averaged for each tissue source (n = 4) and optical densities were normalized to 0–100%.

**Fig. 3.** Comparison of PrP<sub>C</sub> and PrP<sub>Sc</sub> from control and scrapie-infected brains. SDS–PAGE of control and 79A-infected SV mouse brain extracts (lanes 1–4) and control and 263K-infected hamster brain extracts (lanes 5–8). Lanes: 1 and 5; supernatant fluids of control brain (NBr); 2 and 6; supernatant fluids of scrapie-infected brain; 3 and 7, pellets from scrapie-infected brain; 4 and 8, pellets from scrapie-infected brain after proteinase K (PK) digestion. The positions of the H, L and U forms before (e.g. H–PK) and after (e.g. H+PK) PK digestion are shown.

**Fig. 4.** Comparison of PrP<sub>Sc</sub> from different TSE-infected brain areas. SDS–PAGE of PrP<sub>Sc</sub> after proteinase K digestion from cortex (Cx; lanes 1, 4, 7, 10), cerebellum (Cm; 2, 5, 8, 11) and medulla (Ma; 3, 6, 9, 12) from hamster brain infected with the 263K TSE strain (lanes 1–3), SV mouse brains infected with 79A (4–6) or ME7 strains (7–9) and VM mouse brain infected with 301V strain (10–12). Samples were prepared according to the method of Collinge et al. (1996) with 0-1 % 2-mercaptoethanol in the gel sample buffer.
Diversity of PrPSc structure

A wide diversity in PrPSc glycosylation between scrapie strains has been shown previously (Somerville et al., 1997b), showing that TSE strain controls the glycosylation of PrPSc. 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 PrPSc from 79A-infected and ME7- or 22A-infected brains. There was only a small difference between PrPSc 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 PrPSc glycosylation from ME7-infected SV and VM brains.

To determine whether PrPSc glycosylation was under host control, glycoform ratios were determined in cortex, cerebellum and medulla of ME7-infected brains. Significant PrPSc heterogeneity was found between brain regions in this model (Fig. 6), e.g. PrPSc in the cerebellum of brains infected with the ME7 strain was more highly glycosylated than PrPSc from cortex or medulla (Fig. 6c). In brains from VM mice affected with the 301V strain, PrPSc from cerebellum was significantly less glycosylated than that from cortex (% H, P < 0·002) or medulla (% H, P < 0·005) (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 PrPSc 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 PrPSc 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 PrPSc 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. 6a, 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 Sinc7 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 PrPC (Figs 1 and 2), between PrPC and PrPSc (Fig. 3) and for PrPSc (Figs 4 and 6). The glycosylation of PrPSc depends not only on strain of agent (Kacsak et al., 1985; Somerville et al., 1997b) but also on the region of brain and PrP genotype. Recently, it has been reported that no significant PrPSc glycoform differences were detected in different areas of brains infected with three strains of TSE (Kuczius et al., 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 PrPSc from different parts of the brain have previously been observed on silver-stained, two-dimensional Nterminal–SDS gels (Somerville & Ritchie, 1989). Differences have also been found between PrPSc 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).

PrPSc glycosylation from sporadic CJD cases differs according to PrP genotype and clinico-pathological presentation (Parchi et al., 1995), and PrPSc from sporadic CJD cases segregates from PrPSc 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 PrPSc 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 PrPSc diversity

The origin of the differences in glycosylation of PrPSc 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 PrPSc 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 PrPSc 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 PrPSc 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 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 PrPSc and PrPSc 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. PrPSc glycoforms in control and scrapie-infected brain are similar, irrespective of the PrPSc glycoform. Depletion of specific PrPSc glycoforms in scrapie-infected brain would have been evidence of their selection from the mature population of PrPSc. 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 PrPSc to form a PrPSc fraction would require recruitment from the extant PrPSc 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 PrPSc and PrPSc glycosylation. Overall there is no evidence to support the suggestion that PrPSc is selected from the mature PrPSc 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 PrPSc (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 PrPSc between brain regions, and it most readily explains the differences between PrPSc and PrPSc, between PrPSc 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 PrPSc behaved initially like PrPSc (i.e. it was protease K-sensitive and was released by PIPLC) and was located on the cell surface (Caughey & Raymond, 1991). Incorporation of protein into the PrPSc fraction probably occurred at the cell surface; however, the degree of glycosylation of the PrPSc fraction was less than that of the PrPSc 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 PrPSc, 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 PrPSc 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 PrPSc and PrPc glycoforms implies that PrPSc does not originate from in vivo conversion of the mature PrPc 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 PrPSc 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 targetted 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, PrPSc is anchored on the exterior of the plasma membrane and some PrP is released into cell culture medium (Caughey et al., 1993). In scrapie-infected mouse brain, electron microscopic observations suggest that PrP is released into 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 may be synthesized with altered targetting to recipient cell lectins. Assuming that PrP is a component of the agent, this represents a potential mechanism for targetting of agent and hence neuropathological lesions to different cell types and neuronal sub-types. The new PrP population 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 PrPSc. In this model, the TSE agent encodes the information required for strain-specific, host-interactive regulation of glycoform diversity. Such a requirement fits 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).

I thank Orla Mulqueen for technical assistance and many colleagues for their valuable comments on the manuscript.

References


Histochemistry and Cytochemistry

21

the beige mouse: a homologue of Chediak–Higashi syndrome.

proteins.

Protease-resistant prion protein in sporadic


Parchi, P., Castellani, R., Capellari, S., Petersen, R. B., Chen, S. G.,


Molecular weight determination of protein-


Mice with gene targetted prion

Moore, R. C., Hope, J., McBride, P. A., McConnell, I., Seifridge, J.,

Neville, D. M., Jr (1971). Molecular weight determination of protein-


Parekh, R. B., Dwek, R. A., Thomas, J. R., Opdenakker, G., Rade-
macher, T. W., Wittwer, A. J., Howard, S. C., Nelson, R., Siegel, N. R.,


Rubenstein, R., Merz, P. A., Kascik, R. J., Scalici, C. L., Papini, M. C.,


Stahl, N., Baldwin, M. A., Teplow, D. B., Hood, L., Gibson, B. W.,


Received 21 September 1998; Accepted 29 March 1999