Subcellular trafficking abnormalities of a prion protein with a disrupted disulfide loop

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Abstract The single disulfide loop (Cys_{178}-Cys_{213}) of the prion protein (PrP) may stabilize the conformation of this protein by bridging the C-terminal α-helices. The substitution mutant Cys_{178}Ala fails to form the prion isoform PrP^{Sc} when expressed in scrapie-infected neuroblastoma ScN2a cells (Muramoto et al., Proc. Natl. Acad. Sci. USA 93, 15457–15462). To investigate the reasons for this failure, we introduced the C178A substitution in the full length mouse PrP gene as well as in its N-terminally truncated Δ23-88 version. The resulting mutants (C178A and ΔC178A, respectively) were transiently expressed in N2a and CHO cells. Wild-type PrP, wild-type Δ23-88 and the point mutant E199K served as controls in these experiments. Compared to the wild-type controls, the C178A mutants were markedly resistant to proteolysis and they were also vastly insoluble in sarcosyl. Studying the metabolic fate of the C178A mutants, we found that in contrast to control PrP molecules, these mutants (i) remained sensitive to the diagnostic endoglycosidase EndoH, (ii) failed to reach the cell surface and (iii) congregated in large juxtanuclear spots. We surmise that these severe trafficking abnormalities may contribute both to the spontaneous aggregation of the C178A mutants and to their reported inability to form PrP^{Sc}.

Key words: Prion; Misfolding; Thiol; Aggresome; Trafficking

1. Introduction

Prions [1] are proteinaceous agents that cause the transmissible spongiform encephalopathies, which comprise infectious, familial and sporadic diseases. Prions are thought to propagate by refolding a normal glycoprotein of the host, the cellular prion protein (PrP) PrP^{C}, into an abnormal 'prion' conformation (reviewed in [2]). The resulting pathological conformer, PrP^{Sc}, is in turn the only known component of the infectious prion.

In addition to typical prion diseases in which infectious prions propagate, PrP is also involved in metabolic disorders, in which infectious prions have not been demonstrated. Biochemical and cell biological attributes of PrP mutants critically determine their involvement in either type of disorders. For instance, the correct subcellular targeting of PrP^{C} precursors is essential for their subsequent transformation into PrP^{Sc} in prion-infected cells. Hence, preventing PrP^{C} molecules from exiting the ER-Golgi using brefeldin A (BFA) prevented the formation of PrP^{Sc} in scrapie-infected cultured cells [3]. Studies have also shown that mishandling of several mutant PrPs by the cell can lead to storage-like diseases as well as other disorders [4,5]. In addition, several pathogenic mutants that are linked to familial prion diseases in humans display slight metabolic abnormalities when expressed in cultured cells [6].

Mutational analysis coupled with transgenetic experiments have begun to uncover amino acid signals within PrP that are essential for correct PrP^{C} targetting as well as for prion formation and propagation [5,7,8]. Using prion-infected mouse neuroblastoma ScN2a cells, Prusiner and his colleagues [9] recently showed that substituting an alanine for Cys^{178} (mouse PrP numbering, see nomenclature in Section 2), thereby disrupting the unique disulfide loop, abolished the formation of PrP^{Sc}. In mature wild-type (wt) PrP, the cysteines (at positions 178 and 213 of mouse PrP) are both oxidized [10] and the resulting disulfide loop is thought to stabilize the structure of PrP^{C} by linking the two carboxy-terminal helices [11,12]. Cys_{178} neighbors two other prominent residues. First, the immediately adjacent D_{177} (homologous to codon 178 in humans) is substituted by an arginine in several European kindreds suffering from familial CJD [13,14], as well as in kindreds with fatal familial insomnia [15]. Also close to Cys_{178} is the *N*-glycosylation site N_{180}. While glycosylation of PrP is not essential for the formation of protease-resistant PrP^{Sc} [16], under-glycosylated PrP displays various metabolic abnormalities [17,18] and unglycosylated PrP^{C} displays weak 'prion-like' properties [19].

In this paper, we studied the cellular and biochemical properties of PrP with the C178A substitution. To this end, we used the epitopically tagged chimeric PrP designated MHM2-PrP [20]. We substituted an alanine for Cys_{178} in two different PrP platforms: (i) full length MHM2-PrP and (ii) its truncated version Δ23-88 (C178A and ΔC178A, respectively) and transiently expressed these constructs in Chinese hamster ovary (CHO) and N2a cells. We found that both C178A mutants resisted higher levels of proteinase K than wt PrP and were also largely insoluble in sarcosyl. Furthermore, both C178A mutants suffered from profound subcellular trafficking abnormalities, which probably result from their aggregation in the early secretory pathway. We surmise that these abnormalities may contribute to the inability of these mutants to produce PrP^{Sc} in scrapie-infected cells [9].

2. Materials and methods

2.1. Materials

Cell culture reagents were purchased from Biological Industries (Bet Haemek, Israel). Tissue culture plates were obtained from Miniplast (Ein Shemer, Israel) or Nunc (Denmark). G418 and sulfo-NHS biotin (cat #203189) were from Calbiochem (San Diego, CA, USA). Other chemicals were from Sigma (St. Louis, MO, USA). Restriction en-

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zymes were from New England Biolabs (MA) and MBI-Fermentas (Lithuania).

2.2. Nomenclature

The amino acid numbering of the mouse PrP is used throughout this paper. Thus, Cys_{178} in MoPrP corresponds to Cys_{179} in human PrP. MHM2-PrP [20] is a mouse-Syrian hamster chimeric PrP that carries the epitope for the monoclonal antibody (mAb) 3F4 [21]. Here, it is referred to as wt MHM2-PrP. The 65 amino proximal amino acids, which are removed during the trimming of PrP^{Sc} to PrP27-30 [22], are dispensable for the formation of PrP^{Sc} [8,23]. Δ23-88 designates the PrP construct in which these codons have been deleted (Fig. 1).

2.3. Cells and transfections

Mouse neuroblastoma (N2a) and CHO cells were grown at 37°C in DMEM16 (low glucose) supplemented with 10% fetal calf serum. N2a-C10 cells [24] stably express the MHM2-PrP chimera. Transient transfections of the PrP constructs was achieved with the non-liposomal reagent FuGENE 6 (Roche), which was used according to the manufacturer's instructions. Cells were harvested 72 h after the transfection.

2.4. Antibodies

Rabbit antiserum R073 binds to both mouse PrP and to MHM2-PrP [25,26]. MAb 3F4 [21] binds to residues Met_{108} and Met_{111} [27] in chimeric MHM2-PrP but does not recognize the wt mouse PrP endogenous to N2a cells [20]. Both antibodies were used at a dilution of 1:5000 (of the serum or the ascitic fluid, respectively). Secondary antibodies were from Jackson ImmunoResearch (West Grove, PA, USA).

2.5. DNA constructs

In situ mutagenesis was achieved by the method of Kunkel [28] using the primer 5′-TCGTGCACGATGCCGTCAATATCACC. MHM2 was first ligated into pSL301 (Invitrogen, Carlsbad, CA, USA) between the *Sal*I and *Bgl*II sites, then excised with *Eco*RI and *Hin*dIII and ligated into pBluescript II SK+ (Stratagene, La Jolla, CA, USA), where mutagenesis was performed. After confirming the presence of the mutation by sequencing, the mutated gene was excised from pBluescript with *Eco*RI and *Nhe*I and cloned into the expression vector pCI-neo (Promega, Madison, WI, USA). Plasmid DNA for transfections was prepared with the Wizard*Plus* maxiprep DNA purification system (Promega).

2.6. Biochemical analysis of PrP

SDS-PAGE, Western immunoblotting and immunoprecipitation of the PrP isoforms were all carried out as described [24,29]. Cells were lysed in ice-cold 'standard' lysis buffer (0.5% Triton X-100, 0.5% Na-deoxycholate, 150 mM NaCl, 10 mM Tris-Cl, pH 7.5, 10 mM EDTA) and the lysates were immediately centrifuged for 1 min at 14 000 rpm in a microfuge. All biochemical analyses were performed on this post-nuclear fraction. For endoglycosidase H analysis, cell lysates (100 μl) were incubated with the enzyme according to the manufacturer's instructions (EndoH, New England Biolabs, MA, USA).

To assess the degree of aggregation of the various PrP isoforms in cell lysates, we utilized sucrose gradients containing sarcosyl. Assays in sucrose gradients were performed as follows. Cell lysates were loaded on top of pre-formed sucrose step gradients prepared with 300 μl each of 10, 15, 20, 25, 30 and 60% sucrose in TNS (10 mM Tris, pH 7.5, 100 mM NaCl, 1% sarcosyl). The tubes were spun at 55 000 rpm for 1 h at 4°C in a TLS-55 rotor ($g_{av} = 200\,000 \times g$), 180 μl fractions were collected from the top and analyzed as described in each experiment.

2.7. Biotinylation of cell surface proteins

Proteins at the surface of cells were biotinylated as follows. The whole procedure was performed at 10°C. Cells growing in 90 mm petri dishes were rinsed three times in ice-cold buffer A (150 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 20 mM HEPES, pH 7.4) and then incubated for 40 min in buffer A containing 500 μg/ml NHS-X-biotin (water-soluble). The cells were then rinsed three times in ice-cold buffer A supplemented with 50 mM NH_4Cl to quench the biotinylation reagent and then lysed in the standard lysis buffer. Prior to immunoprecipitation, lysates were boiled for 30 min in 2% sarcosyl, diluted 3-fold with TNS, incubated on ice for 60 min and then centrifuged for 1 h at $100\,000 \times g$ to remove insoluble material. Antibody was added to the supernatant for immunoprecipitation, which was carried out as described [16].

2.8. Immunofluorescence

To detect PrP on the surface of cells, we labelled live cells as described [26] to take advantage of the patching effect caused by the cross-linking secondary antibody [30]. Cells were cooled to 4°C and then sequentially incubated with the mAb 3F4, followed by a secondary anti-mouse IgG coupled to FITC (diluted at 1:2000 and 1:200, respectively, in 1% BSA in phosphate-buffered saline (PBS)). Following extensive washes, the cells were fixed with formaldehyde (8% formalin in PBS, 4°C, 15 min) and then quenched by several rinses with 2% NH_4Cl in PBS prior to mounting. To detect total PrP, the cells were fixed (8% formalin in PBS, 4°C, 30 min), rinsed with 2% NH_4Cl in PBS and then permeabilized (0.1% Triton X-100 in PBS, 5 min, room temperature) prior to their incubation with 3F4 and the secondary antibody as described above. In both cases, the labelled cells were mounted in an anti-fading preparation (5% *n*-propyl gallate, 100 mM Tris-Cl, pH 9, 70% glycerol) [31] and viewed in a Zeiss Axioplan microscope equipped with epifluorescence.

3. Results

3.1. PrP-C178A is largely resistant to proteolysis when expressed in uninfected N2a and CHO cells

Previous studies showed that substituting an alanine for Cys_{178} in the truncated Δ23-88 platform, thereby disrupting the disulfide loop, prevents the formation of PrP^{Sc} [9]. To further characterize the properties of PrP with a disrupted disulfide loop, we constructed C178A substitution mutants on two epitopically labelled PrP platforms. First, we used the truncated Δ23-88 version of MHM2-PrP which was previously utilized by Muramoto et al. [9]. Second, since the amino proximal sequence can modulate the prion properties of mutant PrP [4,32], we also substituted an alanine for Cys_{178} in the full length MHM2-PrP gene. These mutants were designated ΔC178A and C178A, respectively (Fig. 1).

We first studied the resistance of the C178A mutants to proteolysis in transiently transfected N2a (Fig. 2A) and CHO (Fig. 2B) cells. As controls for these experiments, we used the wt MHM2-PrP, which behaves as classical PrP^{C}, and the E199K mutant, which displays 'prion-like' properties

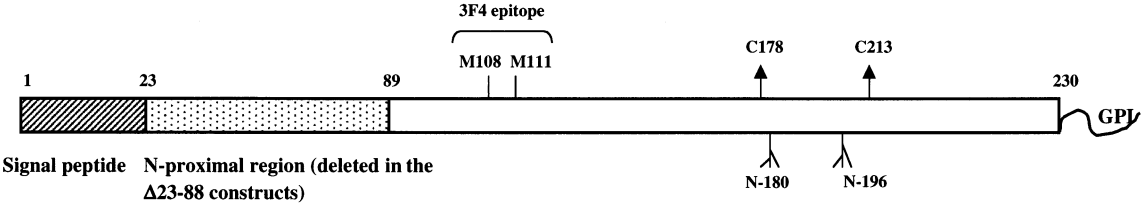

Fig. 1. Schematic depiction of the MHM2-PrP gene. Shown are the *N*-proximal region, the 3F4 epitope, the two cysteines and the two asparagines where *N*-linked sugars are attached.

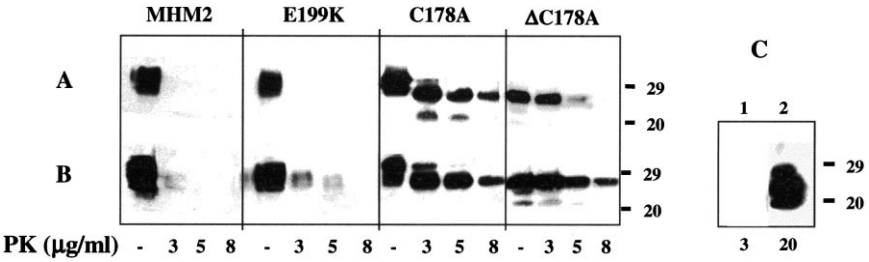

Fig. 2. C178A and ΔC178A are largely protease-resistant in N2a and CHO cells. Wt and mutant PrP constructs, as indicated, were transiently expressed in N2a (A) and CHO (B) cells. Seventy-two h after the transfection, the cells were lysed in standard lysis buffer. Post-nuclear supernatants were digested with proteinase K at the indicated levels (0–8 μg/ml, 30 min, 37°C) and then, the resistant PrP species were detected in Western blots developed with the mAb 3F4. Both C178A mutants were considerably more resistant to proteolysis than the disease-linked mutant E199K. In C, lysates from cells that stably express wt MHM2-PrP were used as controls for these experiments. Lane 1, N2a-C10 cells, digested with 3 μg/ml proteinase K, 30 min, 37°C. Lane 2, ScN2a-C10 cells, digested with 20 μg/ml proteinase K, 1 h, 37°C.

when expressed in CHO cells [6]. Seventy-two h after the transfection, cells were lysed in standard lysis buffer and the post-nuclear supernatants were digested with proteinase K, at the indicated concentrations (0–8 μg/ml, 30 min, 37°C, see Fig. 2). Protease-resistant PrP was then analyzed in Western blots developed with the mAb 3F4, which recognizes MHM2-based PrP molecules. In N2a cells, both wt PrP and the E199K construct were entirely digested at 1 μg/ml (data not shown) and at 3 μg/ml proteinase K (Fig. 2A). Interestingly, however, both constructs were more resistant to proteolysis when expressed in CHO than in N2a cells (compare Fig. 2A,B). In CHO cells, E199K resisted proteolysis more than its wt counterpart (Fig. 2B), consistent with previous results [6].

Surprisingly, sizeable amounts of both C178A and ΔC178A were detected in the cells even following incubation with 8 μg/ml proteinase K and thus, these mutants were both considerably more protease-resistant than the disease-linked E199K mutant [6]. As a control for the proteolytic reaction, we used PrP^{Sc} (which resists even higher levels of proteolysis, 20 μg/ml, 60 min, 37°C) (Fig. 2C, lane 2), as well as PrP^{C} (which is entirely degraded by 3 μg/ml proteinase K) (Fig. 2C, lane 1).

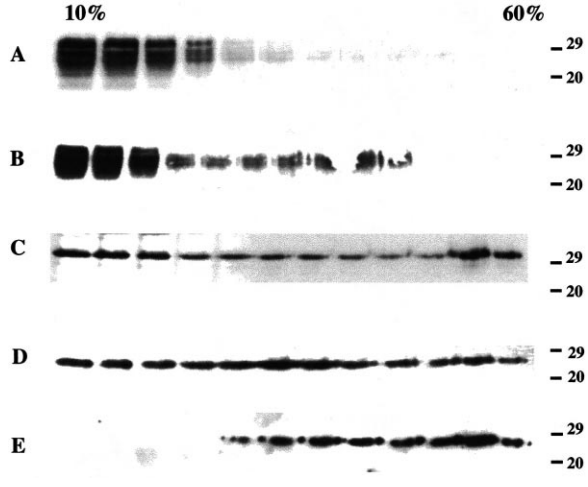

Fig. 3. The C178A mutants are largely insoluble in sarcosyl. Wt MHM2 (A), E199K (B), C178A (C) and ΔC178A (D and E) were transiently expressed in CHO cells. Post-nuclear supernatants were brought to 1% sarcosyl and incubated on ice for 30 min and then analyzed by velocity sedimentation on 10–60% sucrose gradients containing 1% sarcosyl (55 000 rpm, 1 h, 4°C). Gradient fractions were then analyzed for PrP in Western immunoblots developed with 3F4. In C and D, about half of the immunoreactive material was found in the lower half of the gradient. In E, the fractions were incubated with proteinase K (3 μg/ml, 30 min, 37°C) prior to the Western analysis. The ΔC178A molecules found in the heavier fractions resisted proteolysis.

3.2. The C178A mutants are largely aggregated in 1% sarcosyl

Protease-resistance of PrP as well as of other proteins is often accompanied by partial insolubility in detergents. To determine the degree of solubility of the C178A mutants, we used velocity centrifugation through sucrose gradients (Fig. 3). Post-nuclear supernatants of CHO cells expressing MHM2-PrP (Fig. 3A), E199K (Fig. 3B), C178A (Fig. 3C) and ΔC178A (Fig. 3D,E) were incubated with 1% sarcosyl for 30 min on ice and then loaded on top of 10–60% sucrose gradients containing 1% sarcosyl. E199K slightly sedimented through this gradient, contrasting with the MHM2 control which stayed primarily in the top 3–4 fractions of the gradient. This is in line with former results [6] and correlates with the partial protease-resistance of this mutant in CHO cells (see Fig. 2). Interestingly, both C178A mutants sedimented to a much larger extent than E199K, as more than half of the material was found in the bottom fractions of the gradient. The fractions of the ΔC178A gradient shown in Fig. 3D were further analyzed for their resistance to proteolysis. One half of each fraction was treated with proteinase K (3 μg/ml, 30 min, 37°C) prior to the Western blot analysis. Results clearly show (Fig. 3E) that aggregated material indeed resisted proteolysis. Interestingly, the M_r of the C178A mutants was often, but not always, more homogeneous in CHO cells than

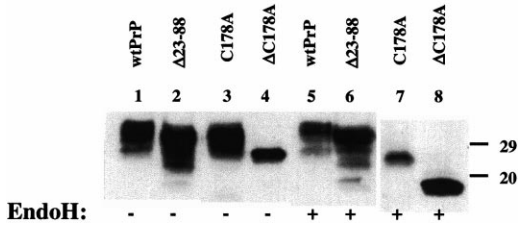

Fig. 4. The C178A mutants are sensitive to EndoH. The wt and mutant PrPs (as indicated) were transiently expressed in N2a cells. Cell lysates were incubated with EndoH or mock-incubated, as indicated, and then analyzed by Western immunoblotting. C178A and ΔC178A were sensitive to EndoH digestion (compare lanes 7 and 8 to lanes 3 and 4).

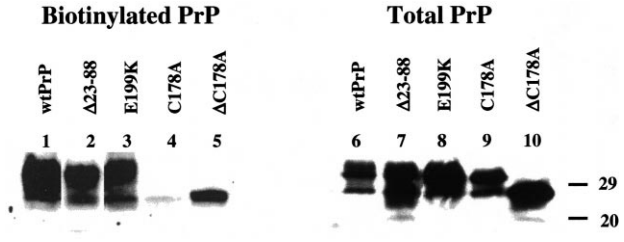

Fig. 5. The C178A mutants fail to reach the cell surface. The various wt and mutant PrPs (as indicated) were transiently expressed in N2a cells. Cell surface was labelled with a membrane-impermeant sulfo-NHS reagent. PrP molecules were immunoprecipitated from half of the lysates using 3F4. The immunoprecipitates were then analyzed in Western blots developed with streptavidin-HRP, to detect biotinylated PrP species (lanes 1–5). The other halves of the lysates were analyzed in Western blots developed with 3F4 (lanes 6–10), demonstrating that all the mutant PrPs were expressed at similar levels. In contrast to the control PrP (lanes 1–3), little, if any, C178A and ΔC178A (lanes 4 and 5, respectively) was biotinylated in this experiment, suggesting that they did not reach the cell surface in large amounts.

in N2a cells, probably reflecting variations in the glycosylation pattern in these cells.

3.3. *In contrast to wt PrP and E199K, both C178A mutants remain sensitive to EndoH and do not reach the cell surface*

To analyze the subcellular localization of the C178A mutants, we first used the diagnostic endoglycosidase EndoH (Fig. 4). Lysates of N2a cells transiently expressing the various PrP constructs were incubated either with or without this endoglycosidase, as indicated in the figure, and then analyzed in Western immunoblots developed with 3F4. We found that while MHM2 and Δ23-88 resisted digestion with EndoH, both C178A mutants were almost entirely digested under these conditions. Thus, the C178A mutants may not cross the mid-Golgi stack. This interpretation was confirmed by cell surface biotinylation experiments (Fig. 5). N2a cells were transiently transfected with the various constructs. Seventy-two h after transfection, the cell surface was labelled in vivo with a membrane-impermeant sulfo-NHS biotin reagent and the cells were then lysed. One half of the lysate was subjected to Western analysis developed with 3F4 (lanes 6–10). This confirmed that the PrP constructs were all expressed at comparable levels. The amount of biotinylated PrP was assessed from the other half of the lysates. Following immunoprecipitation with 3F4, biotinylated PrP molecules were detected on Western blots developed with streptavidin-HRP (lanes 1–5). We found that, while the wt constructs and the E199K mutant were efficiently labelled on the cell surface, little, if any, biotinylated C178A material was detected in these experiments. We thus conclude that the C178A mutants remain primarily in the interior of the cells.

3.4. *Immunofluorescent detection of the C178A mutants in juxtanuclear sites*

To further localize the C178A mutants in the cells, we used immunofluorescence microscopy (Fig. 6). Like most GPI proteins, wt PrP redistributes to form cell surface patches when unfixed cells are incubated with cross-linking antibodies [30]. This property has been used in the past to facilitate the microscopic detection of PrP on the plasma membrane [26,33]. Live CHO cells transiently expressing MHM2 (Fig. 6A,C) or C178A (Fig. 6B,D) were immuno-labelled with 3F4, followed by a secondary antibody conjugated to FITC. As expected, MHM2 formed cell surface patches under these conditions (Fig. 6A), whereas no fluorescence could be detected on cells expressing C178A (Fig. 6B), confirming the results of the biotinylation experiment (Fig. 5). Entirely different results were

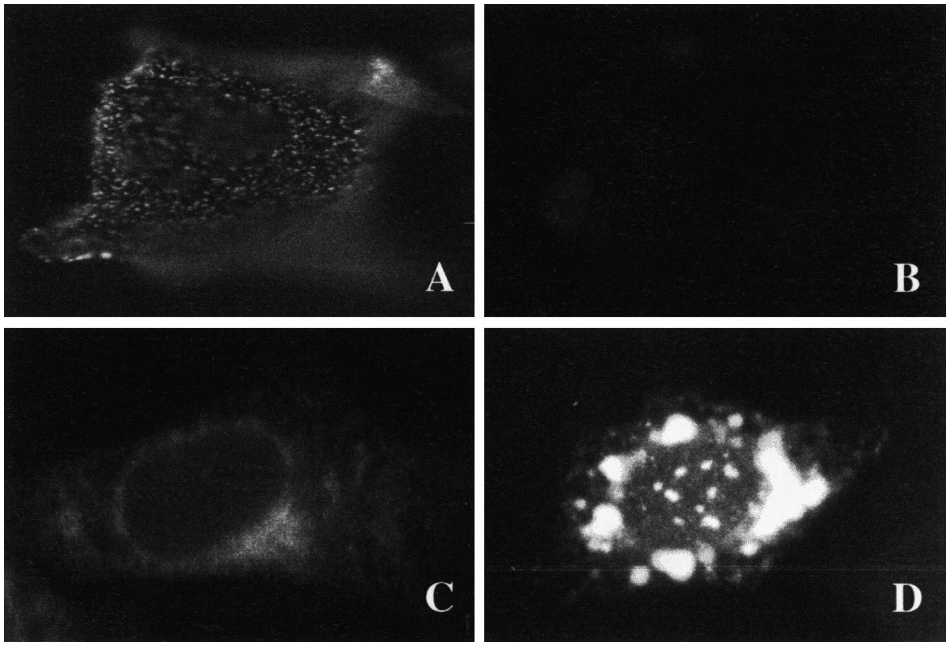

Fig. 6. Cytoplasmic accumulation of C178A. CHO cells transiently expressing MHM2 (A and C) and C178A (B and D) were processed for PrP immunofluorescent detection using the mAb 3F4. A and B: PrP was detected on the surface of live cells using a cross-linking protocol that causes the formation of patches (see A). No PrP was detected on the surface of cells expressing the C178A mutants (B). In C and D, cells were fixed and permeabilized prior to the application of the antibody. The speckled PrP immunoreactivity found in the Golgi region of cells expressing MHM2 (C) contrasted the large juxtanuclear spots found in cells expressing the C178A mutant (D).

obtained when cells were fixed and permeabilized prior to immuno-labelling (Fig. 6C,D). In most cells expressing MHM2, a slight reticulated signal could be detected throughout the cytosol as well as in the Golgi region (Fig. 6C). This immunoreactivity probably represented PrP transitting in the secretory pathway en route for the plasma membrane. In cells expressing C178A, the results were strikingly different (Fig. 6D). In most of these cells, large, strongly immunofluorescent spots were seen. Usually, these spots clustered around the nucleus, but occasionally, they were scattered in more distal locations in the cytosol and even in close proximity to the cell surface. Similar results were also observed in cells expressing ΔC178A (not shown). Pre-incubating the mAb 3F4 with a synthetic peptide including the epitope [27] abolished the immunofluorescent signal, confirming the specificity of this test (data not shown).

4. Discussion

Both C178A substitution constructs suffered from severe trafficking abnormalities when expressed in N2a and CHO cells. They also resisted partial proteolysis and were aggregated in sarcosyl. These metabolic abnormalities may contribute to their previously observed inability to form PrP^{Sc} in scrapie-infected cells [9], perhaps by barring them from reaching the favorable subcellular locations where PrP^{Sc} templates are found and where additional PrP^{Sc} molecules can be manufactured. Trafficking abnormalities have indeed been shown previously to prevent the formation of PrP^{Sc}. When ScN2a cells were treated with BFA, exit of nascent PrP from the ER-Golgi was prevented, its association with detergent-insoluble rafts [34] was suppressed [29] and its transformation into protease-resistant PrP^{Sc} was inhibited [35]. Neither C178A constructs reached the cell surface at detectable levels (Figs. 5 and 6) and their sensitivity to deglycosylation by EndoH indicates that they probably did not traverse the mid-Golgi complex. The association of the C178A mutants with buoyant, detergent-insoluble rafts [24,34,36] was also largely inhibited (A.Y., manuscript in preparation).

The reason for this mislocalization remains to be established. We envisage that the C178A mutants could be retained in the early secretory pathway by at least two mechanisms. First, the exposed thiol of the remaining cysteine could be recognized and actively retained, perhaps by forming disulfide bonds with ER resident proteins. Studies on immunoglobulins revealed that the recognition of exposed thiols is one of the mechanisms that restricts the secretion of unassembled molecules. In the case of Ig-λ chains, retention in the ER was mediated by the COOH-terminal cysteine and correlated with the formation of disulfide bonds with protein disulfide isomerase and ERp72, among other ER proteins [37]. This thiol-mediated retention mechanism was shown to function primarily in the ER. In the case of IgG, it was not operative in or beyond the Golgi complex [38]. In some cases, thiol retention mechanisms could be reversed by treating the cells with a high concentration (ca. 50 mM) of membrane-permeant reducing agents, such as β-mercaptoethanol. Whether such a treatment will be able to alleviate the trafficking block of the C178A mutants remains to be seen. Alternatively, it is also possible that the C178A mutants could be retained in the ER because they do not attain a native conformation, irrespective of thiol retention mechanisms. It is well documented that disulfide bonds help the folding process of proteins and stabilize their native conformation. For instance, native disulfide bonds stabilized both the final conformation of lysozyme and that of early folding intermediates [39].

We do not yet know the nature of the large intracellular structures where the C178A mutants amass. Cells co-stained with an antibody against the ER chaperone BiP showed the conventional reticular pattern of the ER, but no distension (data not shown), suggesting that the C178A mutants did not accumulate within a BiP-containing subcompartment of the ER. An intriguing possibility is that, following their failure to pass the ER folding quality control, the C178A mutants could be dislocated across the ER membrane to the cytosol for proteasomal degradation (ERAD) and that they could accumulate within the cytosol following partial failure of the degradation. Large cytosolic accumulations, located in centrosomal 'aggresomes', were seen following expression of misfolding mutants of the cystic fibrosis transmembrane conductance regulator CFTR and of presenilin-1 in cultured cells [40]. Aggresomal CFTR and PS1 were insoluble in detergents and had a long half-life (more than 24 h) and could be produced either by inhibition of proteasome activity or by its saturation with overexpressed mutants. While a minority of aggresomal protein was ubiquitinated, most possessed a normal molecular weight and thus was not conjugated to ubiquitin. Whether PrP with disrupted disulfide loops accumulates in aggresomes in cells transiently expressing C178A PrPs remains to be seen. In CHO cells, C178A PrP accumulated in more than one juxtanuclear spot.

The possibility that mutant PrP could reside within the cytosol raises intriguing questions. Using ligand blotting, Oesch and coworkers demonstrated that PrP and especially its P5 region (corresponding to amino acids 142–174 of Syrian hamster PrP) binds to the glial intermediate filament molecule GFAP [41]. As long as PrP was thought to be entirely luminal, the implications of this affinity could not be appraised. If PrP was translocated in the cytosol and spared from immediate proteosomal degradation, however, the way would be paved for a functional interaction of PrP with intermediate filaments of glial cells. It will be interesting to see the effect of C178A expression in glial cells. Transmembrane PrP proteins that are not entirely luminal have been described [5,42,43].

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References

[1] Prusiner, S.B. (1982) Science 216, 136–144.
[2] Prusiner, S.B., Scott, M.R., DeArmond, S.J. and Cohen, F.E. (1998) Cell 93, 337–348.
[3] Taraboulos, A., Scott, M., Semenov, A., Avrahami, D. and Prusiner, S.B. (1994) Braz. J. Med. Biol. Res. 27, 303–307.
[4] Chiesa, R., Piccardo, P., Ghetti, B. and Harris, D.A. (1998) Neuron 21, 1339–1351.
[5] Hegde, R.S. et al. (1998) Science 279, 827–834.
[6] Lehmann, S. and Harris, D.A. (1996) J. Biol. Chem. 271, 1633–1637.
[7] Scott, M. et al. (1989) Cell 59, 847–857.
[8] Fischer, M. et al. (1996) EMBO J. 15, 1255–1264.
[9] Muramoto, T., Scott, M., Cohen, F.E. and Prusiner, S.B. (1996) Proc. Natl. Acad. Sci. USA 93, 15457–15462.

[10] Turk, E., Teplow, D.B., Hood, L.E. and Prusiner, S.B. (1988) Eur. J. Biochem. 176, 21–30.
[11] Huang, Z., Gabriel, J.M., Baldwin, M.A., Fletterick, R.J., Prusiner, S.B. and Cohen, F.E. (1994) Proc. Natl. Acad. Sci. USA 91, 7139–7143.
[12] Riek, R., Hornemann, S., Wider, G., Billeter, M., Glockshuber, R. and Wuthrich, K. (1996) Nature 382, 180–182.
[13] Haltia, M., Kovanen, J., Goldfarb, L.G., Brown, P. and Gajdusek, D.C. (1991) Eur. J. Epidemiol. 7, 494–500.
[14] Goldfarb, L.G. et al. (1992) Ann. Neurol. 31, 274–281.
[15] Goldfarb, L.G. et al. (1992) Science 258, 806–808.
[16] Taraboulos, A., Rogers, M., Borchelt, D.R., McKinley, M.P., Scott, M., Serban, D. and Prusiner, S.B. (1990) Proc. Natl. Acad. Sci. USA 87, 8262–8266.
[17] Rogers, M., Taraboulos, A., Scott, M., Groth, D. and Prusiner, S.B. (1990) Glycobiology 1, 101–109.
[18] DeArmond, S.J. et al. (1997) Neuron 19, 1337–1348.
[19] Lehmann, S. and Harris, D.A. (1997) J. Biol. Chem. 272, 21479–21487.
[20] Scott, M.R., Köhler, R., Foster, D. and Prusiner, S.B. (1992) Protein Sci. 1, 986–997.
[21] Kascsak, R.J., Rubenstein, R., Merz, P.A., Tonna-DeMasi, M., Fersko, R., Carp, R.I., Wisniewski, H.M. and Diringer, H. (1987) J. Virol. 61, 3688–3693.
[22] McKinley, M.P., Bolton, D.C. and Prusiner, S.B. (1983) Cell 35, 57–62.
[23] Rogers, M., Yehiely, F., Scott, M. and Prusiner, S.B. (1993) Proc. Natl. Acad. Sci. USA 90, 3182–3186.
[24] Naslavsky, N., Stein, R., Yanai, A., Friedlander, G. and Taraboulos, A. (1997) J. Biol. Chem. 272, 6324–6331.
[25] Serban, D., Taraboulos, A., DeArmond, S.J. and Prusiner, S.B. (1990) Neurology 40, 110–117.
[26] Taraboulos, A., Serban, D. and Prusiner, S.B. (1990) J. Cell Biol. 110, 2117–2132.
[27] Rogers, M., Serban, D., Gyuris, T., Scott, M., Torchia, T. and Prusiner, S.B. (1991) J. Immunol. 147, 3568–3574.
[28] Kunkel, T.A., Roberts, J.D. and Zakour, R.A. (1987) Methods Enzymol. 154, 367–382.
[29] Taraboulos, A., Scott, M., Semenov, A., Avrahami, D., Laszlo, L., Prusiner, S.B. and Avraham, D. (1995) J. Cell Biol. 129, 121–132.
[30] Mayor, S., Rothberg, K.G. and Maxfield, F.R. (1994) Science 264, 1948–1951.
[31] Giloh, H. and Sedat, J.W. (1982) Science 217, 1252–1254.
[32] Goldfarb, L.G., Brown, P., Little, B.W., Cervenáková, L., Kenney, K., Gibbs Jr., C.J. and Gajdusek, D.C. (1993) Neurology 43, 2392–2394.
[33] Stahl, N., Borchelt, D.R., Hsiao, K. and Prusiner, S.B. (1987) Cell 51, 229–240.
[34] Simons, K. and Ikonen, E. (1997) Nature 387, 569–572.
[35] Taraboulos, A., Raeber, A.J., Borchelt, D.R., Serban, D. and Prusiner, S.B. (1992) Mol. Biol. Cell 3, 851–863.
[36] Naslavsky, N., Shmeeda, H., Friedlander, G., Yanai, A., Futerman, A.H., Barenholz, Y. and Taraboulos, A. (1999) J. Biol. Chem. 274, 20763–20771.
[37] Reddy, P., Sparvoli, A., Fagioli, C., Fassina, G. and Sitia, R. (1996) EMBO J. 15, 2077–2085.
[38] Valetti, C. and Sitia, R. (1994) Mol. Biol. Cell 5, 1311–1324.
[39] Goldberg, M.E. and Guillou, Y. (1994) Protein Sci. 3, 883–887.
[40] Johnston, J.A., Ward, C.L. and Kopito, R.R. (1998) J. Cell Biol. 143, 1883–1898.
[41] Oesch, B., Teplow, D.B., Stahl, N., Serban, D., Hood, L.E. and Prusiner, S.B. (1990) Biochemistry 29, 5848–5855.
[42] Hay, B., Barry, R.A., Lieberburg, I., Prusiner, S.B. and Lingappa, V.R. (1987) Mol. Cell. Biol. 7, 914–920.
[43] Lehmann, S. and Harris, D.A. (1995) J. Biol. Chem. 270, 24589–24597.