Molecular Heterosis of Prion Protein β-Oligomers

A POTENTIAL MECHANISM OF HUMAN RESISTANCE TO DISEASE

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The gene encoding prion protein is polymorphic in human populations, with over 40% of native Europeans, for example, being heterozygous for the Met-129 and Val-129 alleles. The polymorphism affects both the incidence and the clinical presentation of a range of prion diseases, with heterozygotes generally showing the highest levels of resistance. It has been suggested that an earlier epidemic of prion diseases exerted balancing selection on the two alleles, and we have previously demonstrated that the two encoded proteins have potentially compensating tendencies to form amyloid and soluble β-oligomers, respectively, in vitro. More strikingly, here we demonstrate that mixed oligomers, composed of both allelic forms, show an extreme sluggishness in converting to amyloid in comparison with oligomers homogenous for either allele. It may be that this example of molecular heterosis in vitro provides the basis for maintenance of the polymorphism in the population and that β-oligomers represent a form of PrP sequestered from pathogenic amyloid formation in vivo.

The human PrP4 gene (PRNP) exists as two common alleles encoding either methionine or valine at codon 129, the former representing the evolutionarily primitive condition found in other mammals (1). The frequency of the Val-129 allele is ~0.3 in Europe, resulting in a frequency of M129V heterozygotes of over 40% (reviewed in Ref. 2). Although the physiological function of PrP, if any, remains obscure, it may be that the maintenance of this polymorphism results from the selective pressure of prion diseases. There is evidence that the polymorphism affects susceptibility to sporadic (3, 4) and acquired (5, 6) prion diseases and of features such as their age of onset and pathological presentation (7–9). Most strikingly, M129V heterozygotes appear to be relatively protected from sporadic, inherited, and infectious prion diseases in these studies. Balancing selection in the face of recurrent prion disease epidemics, such as that experienced by the Fore people of Papua New Guinea (in whom the frequency of heterozygotes is particularly high) has been suggested to account for the maintenance of the polymorphism (10), although recent analysis suggests that this is unlikely to be a general explanation (11). However, until recently, no plausible molecular mechanism to explain these clinical findings could be provided. In particular, the thermodynamic stability of the physiological α-monmeric form of PrP was not affected by the polymorphism (12, 13), nor was its propensity to form amyloid (14, 15).

To address this question, we have investigated the possibility that the polymorphism at position 129 might affect the alternative pathways of in vitro folding of recombinant PrP, schematized in Fig. 1, that were first elegantly demonstrated by Baskakov et al. (16). These authors show that, in mildly denaturing conditions, acidic pH favors the formation of soluble β-oligomers (which we designate βM), whereas neutral pH favors the formation of β-sheet-rich multimers (designated βO, corresponding to multimer I of Ref. 16) that go on to form possibly infectious amyloid fibrils (17). They also show that βO cannot form βM without previously dissociating to a monomeric form (16). Using this system, we have been able to detect potentially significant differences in behavior of the allelo-morphs of human PrP. For example, we showed that the oligomerization pathway (Fig. 1, pathway 2) is favored by the presence of a methionine residue at position 129 (18). Conversely, the amyloid pathway (Fig. 1, pathway 4) is favored by the presence of a valine residue at position 129 (19). In both of these cases, however, the properties of mixtures of Met-129 and Val-129 were intermediate between those of the respective homogenous populations, shedding little light on the presumed behavior of PrP in heterozygous individuals.

In the present work we investigated the possibility that the M129V polymorphism affects pathway 5 (Fig. 1; the formation of βM and hence amyloid from βO). This process is very much slower than the rate of conversion of the physiological form, αM, to amyloid under equivalent conditions. More strikingly, the rate of amyloid formation is more than 5-fold slower from βO comprising an equimolar mixture of Met-129 and Val-129 variants than from homogeneous βO of either variant.
Given the stability of \( \beta^O \) and its resistance to conversion to the self-propagating amyloid form of PrP, we speculate that the entrapment of misfolded PrP in \( \beta^O \), if it were to occur in vivo, might prevent the appearance of \( \beta^M \) and thereby reduce the incidence of prion disease. The peculiar stability of \( \beta^O \), comprising a mixture of the Met-129 and Val-129 forms of PrP, is therefore a form of molecular heterosis that might plausibly provide a basis for selection in vivo that would maintain the polymorphism.

**EXPERIMENTAL PROCEDURES**

**Preparation of Soluble \( \beta \)-Oligomers (\( \beta^O \))**—Expression and purification of the two human PrP variants were done as described previously (18). The formation of \( \beta^O \) was carried out as follows. 1 ml of 6 M guanidine hydrochloride-denatured disulfide-oxidized PrP can be observed under alternative conditions of pH, denaturant, and ionic strength (16, 29). The effect of valine versus methionine at position 129 on the kinetics of pathways numbered 1 to 4 have been reported previously (14, 18, 19); here we report the effect on pathway 5. The relative rate of folding of pure Met-129 PrP (M), Val-129 PrP (V), and equimolar mixtures (MV) are shown for each pathway. The apparently monomeric, transient forms labeled \( \psi^i \) are proposed here to comprise an ensemble of non-native conformations that lie on the interconversion pathway between the \( \beta \)-oligomer (\( \beta^O \)) and multimer (\( \beta^M \)).

**Circular Dichroism (CD)**—CD spectra were recorded in the oligomer buffer (20 mM sodium acetate, 0.2 M NaCl, pH 3.7, 1 M urea, 0.02% sodium azide) using a Jasco-720 spectrometer at room temperature at around 35 \( \mu \)M protein concentration using the following parameters: cell path 0.1 cm, speed 55 nm/s, bandwidth 1.0 nm, resolution 0.5 nm, and response time 4 s. Four individual scans were averaged, and the buffer spectra were subtracted.

**Analytical HPLC**—Reversed-phase high performance liquid chromatography (HPLC) was performed on a C4 column (Sephasil C4, 5 \( \mu \m), 4.6 \times 250 \text{ mm}, 

The elution was done with a linear gradient of \( \text{H}_2\text{O} + 0.1% \text{ trifluoroacetic acid} \) to 95% acetonitrile +0.09% trifluoroacetic acid over 25 min. Size-exclusion HPLC was performed as described above. All HPLC separations were performed at room temperature with a flow rate of 1 ml/min by means of a PerkinElmer HPLC system composed of a binary LC pump 250 and a diode array detector 235C controlled by Total Chrome software, version 6.2 (PerkinElmer Life Sciences), through a PerkinElmer Nelson 600 series link. The eluent was monitored by UV absorption at 280 nm and size-exclusion HPLC. The areas under the peaks were determined with Total Chrome Prism software using nonlinear regression curve fitting.

**Asymmetric Flow, Field-flow Fractionation (FIFFF) and Light Scattering Analysis of \( \beta^O \)**—This was done as described previously (23) using an Eclipse F separation system (Wyatt Technology Europe). The channel was 26.5 cm in length and 350 \( \mu \)m in height, constructed with a trapezoidal spacer of maximal width 21 \( \mu \)m at the inlet, and lined with a 10-kDa cutoff polyether sulfone membrane at the accumulation wall. The sample was loaded in one 20-\( \mu \)l injection and then eluted with 200 \( \mu \)M protein NaCl, 20 \( \mu \)M sodium acetate, pH 3.6, 1 M urea, 0.01% sodium azide at a channel flow of 1 ml/min and a cross-flow of 2 ml/min over 40 min. Static light scattering, refractive index, and dynamic light scattering measurements were carried out on DAWN EOS, Optilab DSP, and WyattQELS instruments, respectively (Wyatt Technologies), and UV absorbance was measured with a Waters 486 tunable absorbance detector. All instruments were connected in line with the FIFFF system. Weight average molar weight (\( M_w \)) and z-average hydrodynamic radius \( \langle r_z \rangle \) values were calculated using ASTRA analysis software (version 4.90.07).

**Amyloid Reaction and Thioflavin T Assay**—A time course study of amyloid formation from the oligomeric forms of recombinant human PrP\(^{90-231} \) allelomorphs was carried out as...
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Preparation and Characterization of Soluble β-Oligomers—All recombinant human PrP forms used in this study were prepared and purified following the procedure that we described previously (16). We prepared soluble β-oligomers (βO) of Met-129, Val-129, and an equimolar mixture of the two by dialysis following the procedure that we described previously (18). We reasoned that these three types of βO should mimic potential PrP states in Met/Met and Val/Val homozygous and Met/Val heterozygous individuals, respectively. Secondly, we used a range of analytical techniques to compare the biophysical properties of the three oligomers (Fig. 2). Size-exclusion HPLC indicated that they had indistinguishable hydrodynamic volume (Fig. 2A). Circular dichroism analysis revealed typical β-sheet spectra with a single minimum at around 218 nm for all three (Fig. 2B).

We had previously shown that oligomers undergo a maturation process during the course of several weeks that can be detected by reversed-phase HPLC. This maturation is more rapid for Met-129 than for Val-129 (18). Accordingly, to ensure that all three oligomers had reached the same maturation stage at the time of the initiation of amyloid reaction, we analyzed them by reversed-phase HPLC (Fig. 2C). All three βO preparations had similar retention times, indicating comparable maturation states.

To determine whether βO comprised a monodisperse population, we used asymmetric FIFFF. This technique was used recently to determine biophysical parameters of the most infectious prion protein particles (23). Oligomers composed of Met-129, Val-129, or both Met-129 and Val-129 eluted as a single peak between 4 and 8 min into the 40-min elution time (see Fig. 2D) in contrast to the broad polydispersity seen with multimeric components of infectious PrPSc (23). Nevertheless, the trailing edge of the oligomer peak indicated the possibility that the fraction might contain more than one species. Accordingly, multi-angle light scattering analysis was used to determine the molecular weight and hydrodynamic radius of each of the species within the peak. The intensity of light scattering depends on both the concentration of monomer and the size of the oligomer, as subunits of an oligomer scatter light coherently. Between 5 and 7 min of elution, corresponding to at least 70% of the oligomer peak, the in-line scattering-based estimate of mass remained flat (see Fig. 2D). This fraction of all three oligomers appeared to have a mass of ~200 kDa by the refractive index method and 210 kDa by the UV method (see Table 1); the latter method is likely the less accurate of the two, given its dependence on a calculated, rather than measured, molar extinction coefficient. This suggests that the bulk of the oligomer comprises 10 subunits, given that the molecular mass of our recombinant PrPs is 19.6 kDa (18), with a minor fraction comprising larger species. The major oligomer species had a hydrodynamic radius of ~5.6 nm (see Table 1). These experiments were conducted on four separate occasions, with essentially identical results.

Conversion from Oligomer to Amyloid Is Delayed by Heterogeneity at Position 129—Having detected no differences in the biophysical properties of the three βO preparations, whether homogeneous or heterogeneous for methionine or valine at position 129, we tested whether they were also equivalent as substrates for amyloid formation. A common feature of amyloid, in contrast with βO, is its ability to bind dyes such as ThT (26). The rapid binding of ThT to amyloid is accompanied by an increase of fluorescence at 482 nm, when excited at 455 nm. Accordingly, we transferred βO to the conditions that favor amyloid formation (namely, moderate denaturation in a pH-neutral buffer with agitation) and monitored the process using the ThT binding assay as described previously (14, 19).

The formation of amyloid from both Met-129 βO and Val-129 βO showed a characteristic nucleation-dependent polymerization pattern, as measured by ThT fluorescence, but with an unusually extended initial lag phase of about 80 h followed by a rapid growth phase (Fig. 3). The absence of any obvious difference in the kinetics of amyloid formation for the two homogeneous oligomers indicated at first that there was no effect of

described previously (16) by monitoring thioflavin T (ThT) fluorescence. Briefly, the oligomers were diluted to a final concentration of 35 μM in phosphate-buffered saline, pH 7.2, containing 3 M urea, 0.02% sodium azide, and a final concentration of 1 M guanidine hydrochloride. Samples were incubated at 37 °C under continuous shaking at 600 rpm on a Delfia plate shaker in 1.5-ml Eppendorf tubes. The kinetics of amyloid formation were analyzed as described previously (19). Formation of amyloid was confirmed by negative stain electron microscopy as described previously (19).

Slot-blot Analysis—The conformationally specific prion aptamer SAF-93 (24, 25) was used under native conditions to probe the conformation of the intermediates species arising from the dissociation of the oligomers during amyloid reaction. The α-monomeric form of PrP was prepared as described previously (14). The individual fractions from size-exclusion HPLC, corresponding to βM, ψ1, and βO (see above and under “Results” for details), were manually collected and immediately applied onto a PVDF membrane. Two hundred pmol of PrPs in each form were applied by vacuum onto a PVDF membrane (0.2 μm pore size) previously equilibrated in 20 mM sodium acetate buffer, pH 4.4, using a Bio-Dot® SF microfiltration apparatus. The membrane was then incubated for 1 h in 15 ml of blocking solution containing 5% Denhardt’s solution in HMNK buffer (20 mM Hepes, pH 7.2, 10 mM MgCl2, 50 mM KCl, 100 mM NaCl) and 20 μg/ml tRNA at room temperature. The blocking solution was replaced with a fresh one supplemented with 700,000 cpm of radiolabeled and refolded SAF-93 or control aptamer from the dissociation of the oligomers during amyloid reaction. The α-monomeric form of PrP was prepared as described previously (18). We reasoned that these three types of ε-mer oligomers indicated at first that there was no effect of
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The polymorphism at codon 129 on this pathway. However, when we mimicked heterozygote condition by initiating amyloid formation from preformed \( \beta^0 \) that contained Met-129 and Val-129 proteins at equimolar ratio, the lag phase of amyloid formation was extended by more than 5-fold to around 450 h (Fig. 3). To test whether this heterosis effect depended on the presence of both Met-129 and Val-129 species within the same oligomer, we repeated the experiment using an equimolar mixture of two preformed, homogeneous \( \beta^0 \) preparations (Fig. 3).

The amyloid reaction showed a lag phase of 110 h, a value very close to that seen during amyloid formation with the two separate, homogeneous \( \beta^0 \) preparations.

These experiments were undertaken on two separate occasions, with four replicates in each experiment. The ThT data were confirmed by extensive negative stain electron microscopy (for illustration, see supplemental Fig. S1). In summary, all samples that were ThT-positive showed the presence of fibrils (of increasing length and topological complexity at greater time points and higher fluorescence), whereas all ThT-negative samples showed no significant structures (data not shown). These results indicate that the effect of the polymorphism at codon 129 is mediated through an interaction between the two allelomorphs within the same oligomer.

Heterogeneous Oligomers Dissociate Slowly—Interestingly, despite the very substantial difference in lag phase, the rate of amyloid growth following nucleation seemed to be very similar (Fig. 3). This suggests that the heterosis effect is entirely restricted to events occurring before nucleation, the most tractable of which is oligomer dissociation. We were able to take advantage of the solubility of \( \beta^0 \) and its well defined elution in size-exclusion HPLC (see Ref. 16) to monitor its behavior during amyloid reaction in parallel with ThT assay (Fig. 4A).

Detailed chromatograms are provided as supplementary data (supplemental Fig. S2).

The dissociation of \( \beta^0 \) to a species with retention time corresponding to monomeric protein (designated \( \psi^0 \)) followed by the appearance of amyloidogenic \( \beta^M \) is consistent with published results (16). Interestingly, the kinetics of dissociation of oligomers appeared to be linear, rather than exponential, and
was almost 100-fold slower for the heterogeneous β⁻⁰ than for either homogeneous β⁻⁰ Figure 4B). Correspondingly, ψ¹ peaked at 2 h in the homogeneous amyloid reactions but only after 430 h in the heterogeneous reaction (Fig. 4C). The pre-amyloid multimer species (β⁻¹) reached half-maximal abundance at between 5 and 6 h in the case of homogeneous reactions but was delayed until about 460 h with the heterogeneous oligomer (Fig. 4D). Nevertheless, following the lag phase, the rate of multimer formation in all reactions was very similar (Fig. 4D).

These experiments were undertaken on two separate occasions, with four replicates in each experi-
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FIGURE 5. Abnormal conformation of transient monomer. The PrP species formed during the $\beta^O$ to amyloid pathway were isolated by size-exclusion HPLC (see Fig. 4); 200-pmol samples were applied to a PVDF membrane in slot-blot format alongside the physiological form, $\alpha^1$. The blots were probed with a $^{32}$P-labeled aptamer, SAF-93, which binds preferentially to abnormally folded forms of PrP (A, upper panel), and with an anti-His antibody, which binds to a tag at the C terminus of all forms (A, lower panel). The radiolabeled aptamer was detected using storage phosphor technology, and the anti-His antibody was detected by chemiluminescence (both using a Storm 840 instrument, GE Healthcare). B, quantification of the background-corrected aptamer SAF-93 binding to the four forms of PrP was done using the volume report function of ImageQuant.

...ment. These results indicate that the rate-limiting step of amyloid formation from $\beta^M$ is the dissociation of $\beta^O$ to $\psi^1$. This dissociation reaction is extremely slow for the mixed oligomer as compared with the two homogeneous oligomers, suggesting that the former is locked in a particularly stable conformation and explaining the heterosis effect noted above.

Is the Transient Monomer Abnormally Folded?—We wished to know whether the apparently monomeric form, $\psi^1$, which transiently appears upon dissociation of $\beta^O$ and which multimerizes to form $\beta^M$, is the same as the physiologically normal form, $\alpha^1$ or is itself abnormally folded. To investigate this question, we took the opportunity to use simple size-exclusion chromatographic fractionation under native conditions to isolate the pathway species, $\beta^O$, $\psi^1$, and $\beta^M$, and investigate their conformation with a structure-sensitive probe. Although formed with very different kinetics from homogeneous versus heterogeneous $\beta^O$ (see above), the chromatographic profiles of the respective $\psi^1$ and $\beta^M$ species were consistent (see Fig. 4A).

We have previously described a 2′F-substituted RNA aptamer, SAF-93, that binds to PrP in a structure-sensitive manner (24, 25). A specific binding site for SAF-93, C-terminal to PrP residue 110, is occluded in the $\alpha$-monomeric, physiological form but exposed in PrPSc and denatured PrP (24). An additional nonspecific nucleic acid-binding site in the N terminus of PrP also binds SAF-93. We used this reagent to probe the conformation of the transient monomer ($\psi^1$) identified above. The amyloid reactions from the homogeneous and heterogeneous oligomers were timed in a way that would allow near simultaneous fractionation of the dissociating intermediates. The corresponding peaks were manually collected and immediately analyzed by slot blot using $^{32}$P-labeled SAF-93 aptamer (Fig. 5A, upper panel). Equal protein loading of each fraction was assured by detection of the C-terminal His tag on all PrP allelomorphs using an anti-His antibody (Fig. 5A, upper panel). Following quantification, no significant allelomorph-dependent differences were detected (data not shown), but the conformation-specific aptamer-binding site was equally exposed in the $\beta^M$ and $\beta^O$ forms (Fig. 5B). As expected, $\alpha^1$ was poorly reactive to SAF-93, but $\psi^1$ showed intermediate SAF-93 aptamer binding (Fig. 5B) that was significantly higher than that of $\alpha^1$ ($p < 0.05$, unpaired $t$ test). The results, obtained in two independent series of experiments, suggest that $\psi^1$ is not in the same conformation as the physiological $\alpha^1$ and retains a degree of misfolding present in the abnormal forms.

DISCUSSION

The elucidation of in vitro folding pathways for recombinant PrP (16) may have allowed the generation of infectious mammalian prions under defined conditions (17). These pathways have also revealed alternative misfolded conformations of PrP and have enabled us and others to shed light on clinically interesting differences between the common allelic forms of human PrP (14, 16, 18, 19, 26–35). However, no mechanistic explanation for the apparent resistance of M129V heterozygotes to prion disease had previously been demonstrated. Moreover, the biological significance of the noninfectious, soluble $\beta$-oligomeric form ($\beta^O$) and its low pH oligomerization pathway (pathway 2 in Fig. 1) was still unclear. We believe that our results shed some light on both of these matters.

It is clear that $\beta^O$ is not directly on the pathway to the formation of $\beta^M$ and amyloid and that $\beta^O$ and $\beta^M$ have distinct conformational properties (16). However, $\beta^O$ has two biophysical properties that are also found in amyloid fibrils, thereby leading to the possibility of confusion between the two species: partial protease K resistance and high $\beta$-sheet content (36, 37). We and others have shown that the protease K cleavage site is around residue 117 in $\beta^O$ but around residue 90 in authentic, infectious PrPSc (18, 38, 39). Previous analysis of $\beta^O$ using mass spectrometry suggested that it was an octamer of PrP (16). However, this was quite possibly an underestimate resulting from dissociation during analysis. Here, we have used in-line light scattering of asymmetric flow field-flow fractions to provide an improved estimate of the predominant molecular mass of $\beta^O$, indicating that it is a decamer. This was a surprising result, as there exist no spherically symmetrical regular solids with 10 vertices. It would, for example, be intuitively straightforward to build oligomers of eight (cubic) or 12 (icosahedral) protomers in which each made equivalent contacts with the other and in which the energetic penalty for adding or removing one protomer would be such as to produce a monodisperse population of defined oligomer. On the other hand, we can rule out the possibility that the $\beta^O$ decamer is a one-dimensional structure (i.e., a string), on both empirical and theoretical grounds. Firstly, the empirical estimate of a 6-nm radius for $\beta^O$ is much too small to fit 10 plausible PrP protomers in a linear arrangement. Second, one would need to posit a special mechanism for terminating the elongation of the oligomer at exactly the tenth protomer. We speculate, therefore, that the decamer is most likely to be an essentially two-dimensional structure with radial symmetry,
such as a 10-segmented ring or disk as we indicate in the schematic (Fig. 1). Accordingly, we have attempted to elucidate the quaternary structure of \( \beta^O \) using both NMR and crystallographic methods.

It has recently been demonstrated that \textit{in vitro} produced PrP amyloid and \( \beta^O \) are acutely toxic to PrP-expressing neuronal cells in culture, whereas \( \alpha^1 \) is benign (40). Despite its potential neurotoxicity, \( \beta^O \) might represent a physiologically adaptive conformer for PrP, for three reasons. First, its quaternary organization is characteristically stable. That is, a \( \beta^O \) decamer does not grow progressively, whereas \( \beta^M \) and amyloid propagate indefinitely. Second, \( \beta^O \) is perfectly soluble at very high concentrations, enabling it to be cleared by lymphatic drainage to the reticuloendothelial system. Third, \( \beta^O \) acts as a trap for misfolded monomeric forms of PrP, such as \( \psi^1 \), preventing them from entering the pathway of amyloid formation. In the light of this interpretation, the extraordinary stability of \( \beta^O \) composed of a mixture of Met-129 and Val-129 PrP variants, as would be expected to form in M129V heterozygous individuals, is highly suggestive. A full, mechanistic explanation for this stability awaits the elucidation of the structure of the \( \beta^O \) decamer (see comments above), but it is tempting to predict that it will reveal additional atomic interactions between Met-129 and Val-129 protomers within the mixed decamer that are absent in the homogeneous assembly. We have been unable to show any differences in the denaturant concentration threshold for disruption of \( \beta^O \) among homogeneous and mixed oligomers, suggesting that the energetic differences of interprotomer interaction are relatively small.

The apparently monomeric, transient form of PrP (\( \psi^1 \)) is clearly not the same as the physiological \( \alpha^1 \) form. Although \( \psi^1 \) is ephemeral, preventing us from analyzing it by CD, we were able to isolate enough of it to probe it using structure-sensitive probes such as aptamers and thereby test the hypothesis outlined above. As oligomerization is highly concentration-dependent, transgenic mice that over-express human PrP alleles might provide a suitable system for this sort of approach.

It might reasonably be argued that the conditions used to produce \( \beta^O \) \textit{in vitro} are sufficiently nonphysiological that it would be unlikely to have relevance to the pathogenesis of prion diseases. We feel, however, on balance that the stability, solubility, and order of this form are consistent with its having an adaptive function and that they motivate one to ask whether conditions \textit{in vivo} might not, in fact, be conducive to its formation. Although unproven, we don’t think the possibility can be excluded that PrP might reach local concentrations \textit{in vivo} (for example in lipid “rafts”) under conditions of sufficiently low pH (such as lysosomal vesicles), and in the presence of structure-destabilizing molecules (such as chaperones), that oligomerization could occur at rates that are relevant in the context of the kinetics of prion diseases.

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REFERENCES

1. Owen, F., Poulter, M., Collinge, J., and Crow, T. J. (1990) \textit{Am. J. Hum. Genet.} \textbf{46}, 1215–1216
2. Mitrova, E., Mayer, V., Jovankovicova, V., Silvarichova, D., and Wsolovak, L. (2005) \textit{Eur. J. Neurol.} \textbf{12}, 998–1001
3. Palmer, M. S., Dryden, A. J., Hughes, J. T., and Collinge, J. (1991) \textit{Nature} \textbf{352}, 340–342
4. Laplanche, J. L., Delasnerie-Laupretre, N., Brandel, J. P., Chatelain, J., Beaudry, P., Alperovitch, A., and Launay, J. M. (1994) \textit{Neurology} \textbf{44}, 2347–2351

Clement Mitchell, personal communication.

5. Brown, P., Cervenakova, L., Goldfarb, L. G., McCombie, W. R., Rubenstein, R., Will, R. G., Pocchiari, M., Martinez-Lage, J. F., Scalici, C., Masullo, C., Graupera, G., Ligan, J., and Gajdusek, D. C. (1994) \textit{Neurology} \textbf{44}, 291–293

6. Collinge, J., Palmer, M. S., and Dryden, A. J. (1991) \textit{Lancet} \textbf{337}, 1441–1442

7. Poulter, M., Baker, H. F., Frith, C. D., Leach, M., Lofthouse, R., Ridley, R. M., Shah, T., Owen, F., Collinge, J., Brown, J., et al. (1992) \textit{Brain} \textbf{115}, 675–685

8. MacDonald, S. T., Sutherland, K., and Ironside, J. W. (1996) \textit{Neuropathol. Appl. Neurobiol.} \textbf{22}, 285–292

9. Kovacs, G. G., Head, M. W., Bunn, T., Laszlo, L., Will, R. G., and Ironside, J. W. (2000) \textit{Neuropathol. Appl. Neurobiol.} \textbf{26}, 463–472

10. Mead, S., Stumpf, M. P., Whitfield, J., Beck, J. A., Poulter, M., Campbell, T., Uphill, J. B., Goldstein, D., Alpers, M., Fisher, E. M., and Collinge, J. (2003) \textit{Science} \textbf{300}, 640–643

11. Soldevila, M., Calafell, F., Helgason, A., Stefansson, K., and Bertranpetit, J. (2005) \textit{Trends Genet.} \textbf{21}, 389–391

12. Liemann, S., and Glockshuber, R. (1999) \textit{Biochemistry} \textbf{38}, 3258–3267

13. Hosszu, L. L., Jackson, G. S., Trevitt, C. R., Jones, S., Batchelor, M., Bhelt, D., Prodomidou, K., Clarke, A. R., Waltho, J. P., and Collinge, J. (2004) \textit{J. Biol. Chem.} \textbf{279}, 28515–28521

14. Tahiri-Alaoui, A., and James, W. (2005) \textit{Protein Sci.} \textbf{14}, 942–947

15. Apetri, A. C., Vanik, D. L., and Surewicz, W. K. (2004) \textit{Biochemistry} \textbf{44}, 15880–15888

16. Baskakov, I. V., Legname, G., Baldwin, M. A., Prusiner, S. B., and Cohen, F. E. (2002) \textit{J. Biol. Chem.} \textbf{277}, 21140–21148

17. Legname, G., Baskakov, I. V., Nguyen, H. O., Riesner, D., Cohen, F. E., DeArmond, S. J., and Prusiner, S. B. (2004) \textit{Science} \textbf{305}, 673–676

18. Tahiri-Alaoui, A., Gill, A. C., Disterer, P., and James, W. (2004) \textit{J. Biol. Chem.} \textbf{279}, 31390–31397

19. Baskakov, I. V., Breyo, L., Shaw, M., Gill, A., James, W., and Tahiri-Alaoui, A. (2005) \textit{FEBS Lett.} \textbf{579}, 2589–2596

20. Jackson, G. S., Hosszu, L. L., Power, A., Hill, A. F., Kenney, J., Saibil, H., Craven, C. J., Waltho, J. P., Clarke, A. R., and Collinge, J. (1999) \textit{Science} \textbf{283}, 1935–1937

21. Tattum, M. H., Cohen-Krausz, S., Khalili-Shirazi, A., Jackson, G. S.
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Orlova, E. V., Collinge, J., Clarke, A. R., and Saibil, H. R. (2006) J. Mol. Biol. 357, 975
22. Welker, E., Raymond, L. D., Scheraga, H. A., and Caughey, B. (2002) J. Biol. Chem. 277, 33477–33481
23. Silveira, J. R., Raymond, G. J., Hughson, A. G., Race, R. E., Sim, V. L., Hayes, S. F., and Caughey, B. (2005) Nature 437, 257–261
24. Rhie, A., Kirby, L., Sayer, N., Wellesley, R., Disterer, P., Sylvester, I., Gill, A., Hope, J., James, W., and Tahiri-Alaoui, A. (2003) J. Biol. Chem. 278, 39697–39705
25. Sayer, N. M., Cubin, M., Rhie, A., Bullock, M., Tahiri-Alaoui, A., and James, W. (2004) J. Biol. Chem. 279, 13102–13109
26. Baskakov, I. V., Legname, G., Gryczynski, Z., and Prusiner, S. B. (2004) Protein Sci. 13, 586–595
27. Baskakov, I. V., Aagaard, C., Mehilhorn, I., Wille, H., Groth, D., Baldwin, M. A., Prusiner, S. B., and Cohen, F. E. (2000) Biochemistry 39, 2792–2804
28. Swietnicki, W., Morillas, M., Chen, S. G., Gambetti, P., and Surewicz, W. K. (2000) Biochemistry 39, 424–431
29. Baskakov, I. V., Legname, G., Prusiner, S. B., and Cohen, F. E. (2001) J. Biol. Chem. 276, 19687–19690
30. Lu, B. Y., and Chang, I. Y. (2001) Biochemistry 40, 13390–13396
31. Morillas, M., Vanik, D. L., and Surewicz, W. K. (2001) Biochemistry 40, 6982–6987
32. Sokolowski, F., Modler, A. J., Masuch, R., Zirwer, D., Baier, M., Lutsch, G., Moss, D. A., Gast, K., and Naumann, D. (2003) J. Biol. Chem. 278, 40481–40492
33. Rezaei, H., Eghiaian, F., Perez, J., Doublet, B., Choiset, Y., Haertle, T., and Grosclaude, J. (2005) J. Mol. Biol. 347, 665–679
34. Bocharova, O. V., Breydo, L., Parfenov, A. S., Salnikov, V. V., and Baskakov, I. V. (2005) J. Mol. Biol. 346, 645–659
35. Vendrely, C., Valadie, H., Bednarova, L., Cardin, L., Pasdeloup, M., Cappadoro, J., Bednar, J., Rinaudo, M., and Jamin, M. (2005) Biochim. Biophys. Acta 1724, 355–366
36. Calzolai, L., and Zahn, R. (2003) J. Biol. Chem. 278, 35592–35596
37. Rezaei, H., Choiset, Y., Eghiaian, F., Treguer, E., Mentre, P., Debey, P., Grosclaude, J., and Haertle, T. (2002) J. Mol. Biol. 322, 799–814
38. Qin, K., Yang, D. S., Yang, Y., Chishti, M. A., Meng, L. J., Kretzschmar, H. A., Yip, C. M., Fraser, P. E., and Westaway, D. (2000) J. Biol. Chem. 275, 19121–19131
39. Oesch, B., Westaway, D., Walchli, M., McKinley, M. P., Kent, S. B., Aebersold, R., Barry, R. A., Tempest, P., Teplow, D. B., Hood, L. E., Prusiner, S., and Weissmann, C. (1985) Cell 40, 735–746
40. Novitskaya, V., Bocharova, O. V., Bronstein, I., and Baskakov, I. V. (2006) J. Biol. Chem.
41. Safar, J., Roller, P. P., Gajdusek, D. C., and Gibbs, C. J., Jr. (1994) Biochemistry 33, 8375–8383