The [URE3] Yeast Prion Results from Protein Aggregates That Differ from Amyloid Filaments Formed in Vitro*

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Leslie Ripaud†, Laurent Maillet, Françoise Immel-Torterotot, Fabien Durand, and Christophe Cullin‡

From the Institut de Biochimie et Génétique Cellulaires, 1, rue Camille Saint Saëns, UMR 5095, CNRS Université Bordeaux 2 Victor Segalen, 33077 Bordeaux cedex France

The [URE3] yeast prion is a self-propagating inactive form of the Ure2 protein. Ure2p is composed of two domains, residues 1–93, the prion-forming domain, and the remaining C-terminal part of the protein, which forms the functional domain involved in nitrogen catabolite repression. In vitro, Ure2p forms amyloid filaments that have been proposed to be the aggregated prion form found in vivo. Here we showed that the biochemical characteristics of these two species differ. Protease digestions of Ure2p filaments and soluble Ure2p are comparable when analyzed by Coomassie staining as by Western blot. However, this finding does not explain the pattern specifically observed in [URE3] strains. Antibodies raised against the C-terminal part of Ure2p revealed the existence of proteolysis sites efficiently cleaved when [URE3], but not wild-type crude extracts, were submitted to limited proteolysis. The same antibodies lead to an equivalent digestion pattern when recombinant Ure2p (either soluble or amyloid) was analyzed in the same way. These results strongly suggest that aggregated Ure2p in [URE3] yeast cells is different from the amyloid filaments generated in vitro.

Prion proteins are misfolded proteins that propagate through their interaction with the normal (correctly folded) host protein. The prion concept, initially launched in 1967 (1) and clearly established in 1982 (2), is the result of several decades of studies related to mammalian transmissible spongiform encephalopathies. The prion protein involved in this pathological process is PrP. The cellular isoform PrPc consists mainly of α-helices, whereas its pathological isoform PrPSc represents predominantly β-sheets (3). PrPSc is prone to aggregation and is protease-resistant. Only very recently, protease-resistant PrP aggregates generated in vitro have been reported to be infectious (4). However, the exact nature of the infectious agent remains unknown.

The yeast Saccharomyces cerevisiae contains several proteins with prion-like properties (5–7). Because the "prionization" mechanism has been conserved through evolution (8), they are highly tractable models for studying the mechanisms of prion propagation. The most extensively studied yeast prion proteins are Ure2p and Sup35p, responsible for the [URE3] and [PSI] phenotypes, respectively. Ure2p is a regulator of nitrogen catabolite repression (NCR) sensitive transcription in S. cerevisiae (9, 10). In the presence of a good nitrogen source (asparagine, ammonia, or glutamine) Ure2p complexes with the Gln3p transcription factor in the cytoplasm (11, 12), thus preventing the transcription of genes expressed upon nitrogen starvation. One of these genes is DAL5, which encodes a peroxisome for ureidosuccinate (Usa) (13). As the prion isoform of Ure2p is inactive, DAL5 is expressed in [URE3] cells, and the yeast are capable of importing Usa thus permitting the growth in selective medium (14). Sup35p is a component of the translation termination factor in S. cerevisiae (15). [PSI] prion causes partial read through of stop codons (16). These two proteins have an asparagine/glutamine-rich N-terminal domain, called the prion-forming domain (PFD), which is necessary and sufficient to propagate the prion state.

The nature of the conformational changes between the cellular and the prion isoforms of these proteins was largely investigated but still remains unclear. The self-propagating species especially, called prion, has not been yet characterized. A clear link exists between the yeast prion mechanism and the aggregation of the protein involved in this process. The [PSI] model is the most documented. In [PSI] yeast cells, Sup35p aggregates and is found in the pellet fraction in sedimentation assays (17, 18). The Sup35-green fluorescent protein fusion protein forms several foci in yeast cells that bear [PSI] (18). Moreover, Sup35p forms amyloid fibrils in vitro (19), which are infectious and recapitulate all strain properties when introduced in yeast cells (20, 21).

Like Sup35p, Ure2p has similar properties of assembly into amyloid fibrils in vitro (22). In a test tube, the N-terminal PFD connects each subunit and forms an amyloid filament backbone surrounded by the C-terminal moieties (23). However, the role of aggregation in [URE3] prion propagation is controversial. Ure2-green fluorescent protein fusion proteins only form foci in [URE3] yeast cells (24) under experimental conditions leading to the elimination of the prion (25–27). Otherwise the fluorescence remains diffuse. Sedimentation assays also led to contradictory results. Although Schlumberger et al. (28) observed increased aggregation in [URE3] cells, the only difference we noticed is a decrease in Ure2p concentration measured in a Western blot of a crude extract of the [URE3] strains (27).

In this work we have investigated these discrepancies. We observed that Ure2p is actually aggregated in [URE3] cells. These aggregates behave differently from the Sup35p aggregates found in [PSI]-containing cells. The Ure2p aggregates are resistant to boiling in 2% SDS buffer and need strong denaturing agents to be detected on Western blots. We determined the

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‡ To whom correspondence should be addressed. Tel.: 33-556-999-017; Fax: 33-556-999-017; E-mail: Christophe.Cullin@ibgc.u-bordeaux2.fr.

1 The abbreviations used are: NCR, nitrogen catabolite repression; Usa, ureidosuccinate; PFD, prion-forming domain.
amount of Ure2p required to give a wild-type phenotype and concluded that in [URE3] yeast cells, the aggregated protein should be inactive. We further characterized [URE3] aggregates by limited proteolysis using antibodies raised against the full-length protein. We could definitively confirm that Ure2p is not proteolysed in the same way in [URE3] or in wild-type yeast cells. Next, we used antibodies raised against the C-terminal part of Ure2p to analyze the proteolyzed species. As the proteolysis pattern of Ure2p in [URE3] is not consistent with the amyloid pattern of Ure2p [23, 29], we produced both soluble and amyloid Ure2p and compared their proteolysis pattern under the same experimental conditions. We concluded that the process in [URE3] leading to Ure2p aggregates differs from the process leading to amyloid fibers formed in a test tube.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Media, and Plasmids**—The following strains of *S. cerevisiae* were used in this study. CC30 genotype is *trpl-1, ade2-1, leuc2-3,112, his3-11,15, ura2::HIS3*. The CC34 [URE3] strain is isogenic to CC30. The CC32 genotype is *trpl-1, ade2-1, leuc2-3,112, his3-11,15, ura2::HIS3, cy2::, aux2::CYP2*. Yeast transformations were carried out as described previously [30]. Unless otherwise specified, yeasts were grown in YPD (1% bactoyeast extract, 1% bactopeptone, 2% dextrose, 20 mg/mliter adenine). SD medium (0.67% yeast nitrogen base, 2% dextrose) supplemented with 20 mg/liter adenine, 20 mg/liter tryptophan, and 60 mg/liter leucine. Either 20 mg/liter uracil or 15 mg/liter ureido-succinate was added as specified in the text.

The pYe2L-URE2 BamHI-HpaI fragment containing the Ure2 open reading frame was cloned into the SmaI site of the pET3a expression vector (Novagen). A His6 tag was PCR-amplified and inserted at the 3′-end of *URE2* resulting in pET3a-URE2tagHIS. The protein expression in pET3a-URE2tagHIS was not detected after boiling in 2% SDS buffer. To analyze the proteolysis pattern under the same experimental conditions. We concluded that this antibody was affinity-purified, and the specificity of this antibody was checked. No signal was detected in cells expressing a Ure2 protein truncated from the C-terminal domain (data not shown).

**Protein Extraction, Sedimentation Analysis, and Western Blotting**—Total yeast protein extracts were prepared by glass bead disruption in TNE buffer (50 mM Tris-Cl, pH 7.4, 100 mM NaCl, 0.2% Triton X-100). Samples were boiled for 5 min in loading buffer (0.06 M Tris-Cl, pH 6.8, 2% SDS, 10% glycerol, 5% b-mercaptoethanol, 0.002% bromphenol blue). Urea was added to an 8 M final concentration in all samples unless otherwise specified. Proteins were separated by 12% SDS-PAGE. Gels were stained with Coomassie blue, or used in Western blots to transfer proteins to a nitrocellulose membrane (Optitran BA-S83, Schleicher & Schuell). Membranes were probed with specific affinity-purified polyclonal antibodies raised against full-length Ure2p, against an Ure2p peptide corresponding to the C-terminal domain (data not shown).

**Antibodies Production and Purification**—The AGTPFMQSR-MRFY peptide corresponding to the Ure2p sequence from amino acids 284 to 298 was synthesized in the form of a carboxamide end coupled to hemocyanin. Rabbit antiserum was affinity-purified, and the specificity of this antibody was checked. No signal was detected in cells expressing a Ure2 protein truncated from the C-terminal domain (data not shown).

**RESULTS**

**Urea Enhances Ure2p Detection from [URE3] Extracts in Western Blots**—In a previous work, we found that the Ure2p signal intensity in Western blot experiments was very low in the [URE3] strain compared with the wild-type strain. This may be because of a lower Ure2p concentration in a prion-containing cell, or alternatively to a lower detection efficiency. The effect of denaturing agents like urea already used by others (28) was then tested. With our previous standard protocol we observed that the Ure2p signal intensity was 10 times lower in the CC34 [URE3] strain than in CC30 [ure3-0] (27). When urea was added to a final concentration of 8 M in the loading buffer, a 5-fold increase of this signal was observed (data not shown). This increase shows that most Ure2p [URE3] was not detected after boiling in 2% SDS buffer. To analyze whether the urea-sensitive fraction was soluble or aggregated, a 100,000 × g sedimentation assay was performed. Without urea, most Ure2p was detected in the supernatant fraction, whereas the proteins were prepared from the wild-type or the [URE3] strains (Fig. 1A). Addition of urea after fractionation had no effect on the signal detected in wild-type extracts (Fig. 1A, wt, columns S and P). The intensity of the Ure2p species remained the same in both the pellet and the supernatant. In [URE3] extracts, the supernatant fraction exhibited the same intensity whether urea was added or not (Fig. 1A, [URE3], column S), whereas in the pellet fraction (Fig. 1A, [URE3], column P) the presence of urea strongly increased the detected signal. Thus, most Ure2p is aggregated in [URE3] cells. Without urea, this fraction (representing 80% of total Ure2p) is not efficiently detected on Western blot. In a previous work, Schlumberger et al. (28) found that Ure2p was completely insoluble in [URE3] strains, whereas we found that even in [URE3] 20% remains soluble. The difference in the level of aggregation is in fact because of growth conditions. When CC34 [URE3] cells were grown overnight (5–7 generations) in rich medium YPD, 80% of the Ure2p signal was detected in the pellet fraction (Fig. 1B and C). When the same strain was grown in SD + Usa (accordingly to the conditions used by Schlumberger et al. (28)), no signal was detected in the supernatant (Fig. 1B, B and C) indicating a complete aggregation of Ure2p. This result was also found for another yeast strain (Fig. 1C), indicating that this rather represents a general feature being independent of the genetic background of our strain. The SD + Usa medium is selective for the presence of [URE3] prion. However, decrease in Ure2p aggregation in non-selective medium could not be explained by a partial [URE3] curing in the population, because all the yeast cells remained [URE3] after growth in YPD (data not shown). As the SD + Usa medium is selective for the loss of function of Ure2p, enhanced aggregation of this protein in this medium might be because of a need
of its complete inactivation. To test for this hypothesis, we investigated which amount of Ure2p was required for the wild-type phenotype (absence of growth in SD + Usa).

Ure2p Quantity Sufficient to Inhibit Usa Uptake—The URE2 open reading frame was cloned upstream of a tetracycline-repressible promoter in the pCM183/URE2 plasmid. This plasmid was transformed into the CC32 strain, creating pCM183/URE2. CC32 (ure2Δ) transformants with pCM183/URE2 are noted \(PTET-URE2\). In this strain Ure2p is under the control of the TET-repressible promoter. The CC30 (\(\text{ure2}\Delta\)) and \(PTET-URE2\) were grown overnight in liquid medium SD + uracil and doxycycline ranging from 0 to 2 \(\mu\)g/ml. A, then an aliquot of each culture was used to quantify Ure2p production by Western blot analysis. An antibody against Pap1p was used to normalize the quantification. The Ure2p level was finally obtained with 0.3 \(\mu\)g/ml of doxycycline, leads to an incomplete complementation phenotype (Fig. 2, A and B, column 4). The growth on SD + Usa is heterogeneous. Using 2 \(\mu\)g/ml of doxycycline, the Ure2p level is about 15% of the wild-type level. In this condition, yeast cells clearly grow on SD + Usa as the ure2Δ control (Fig. 2B, columns 5 and 6). These results suggest that less than 15% of Ure2p (in comparison to the wild-type level) gives rise to a mutant phenotype. If aggregation leads to Ure2p inactivation, then a complete aggregation of Ure2p is not required to obtain the mutant [URE3] phenotype. We next investigated the biochemical nature of the aggregated form of Ure2p in [URE3] cells.

Ure2p\(^{\text{P}}\) and Ure2p\(^{\text{URE3}}\)-limited Proteolysis—We further characterized these aggregates by limited proteolysis. This is a tool to investigate protein structure, as flexible regions are more sensitive to proteolysis than folded polypeptide chains. Moreover, the core of ordered aggregates has a lower accessibility to proteases and is expected to be very resistant to proteolysis. Proteins extracted from wild-type and [URE3] strains were digested for 30 min with proteinase K concentrations ranging from 0 to 15 \(\mu\)g/ml. As proteins were extracted and incubated without protease inhibitors for 30 min, each sample
was analyzed by Coomassie staining to ensure that proteins were not degraded in the control without proteinase K (Fig. 3A). Then, Western blots were performed with antibodies raised against Ure2p. In wild-type extracts, Ure2p was completely digested after a treatment with 10 μg/ml of proteinase K (Fig. 3B, wt blot). In contrast, full-length Ure2p was still detected with 15 μg/ml of proteinase K in [URE3] extracts (Fig. 3B, [URE3] blot). Partial degradation of Ure2p from wild-type extracts with 1 μg/ml of proteinase K produced a fuzzy band at 30 kDa, probably corresponding to several fragments arising from different cleavage sites. With higher proteinase K concentration (4 μg/ml), one additional faint band at 25 kDa was observed. When [URE3] crude extracts were analyzed in the same way, a 30-kDa fragment was observed with concentrations at 37 °C for 30 min. Samples were analyzed by SDS-PAGE with Coomassie staining. B, samples were then analyzed on a Western blot probed with an antibody raised against full-length Ure2p. The numbers on the vertical axis denote the masses of marker proteins (kDa). wt, wild-type.

**Fig. 3.** Limited proteolysis of Ure2p cellular and prion isoforms. A, total protein extracts were prepared from wild-type and [URE3] strains. They were digested with increasing proteinase K concentrations at 37 °C for 30 min. Samples were analyzed by SDS-PAGE with Coomassie staining. B, samples were then analyzed on a Western blot probed with an antibody raised against full-length Ure2p. The numbers on the vertical axis denote the masses of marker proteins (kDa).

To characterize which domains of Ure2p had an increased resistance to proteolysis, an antibody raised against a peptide located in the C-terminal domain of Ure2p, from amino acids 284 to 298, was used to probe the Western blots. In [URE3] extracts, full-length Ure2p was revealed by the “C-terminal” antibody, but none of the three bands in the range of 26–30 kDa was detected (Fig. 4A, Cter-Ure2p). When the same membrane was incubated with anti-Ure2p antibodies raised against the full-length protein, the full-length Ure2p and the 26–30-kDa fragments were detected (Fig. 4A, Ure2p). As the fuzzy 30-kDa species generated by controlled proteolysis of wild-type crude extract are recognized by the anti-C-terminal antibodies (Fig. 4B), we concluded that the more accessible proteinase K sites of Ure2p in [URE3] cells are different from Ure2p in wild-type.

**Limited Proteolysis for Recombinant Soluble Ure2p and Ure2p Amyloid Fibrils—**To test whether aggregated Ure2p ([URE3]) was compatible with an amyloid fibril conformation or not, proteolysis patterns of Ure2p extracted from yeast cells were compared with the ones of recombinant soluble Ure2p and Ure2p amyloid fibrils.

Ure2p-His₆ was expressed and purified from E. coli. Amyloid fibrils formation was checked using negative staining electron microscopy (Fig. 5A). We first tested the influence of temperature and urea on the solubility of Ure2p. As expected, neither boiling nor adding urea enhanced the level of protein detected by Coomassie staining of SDS-PAGE when the soluble protein was analyzed (data not shown). On the other hand, when the amyloid sample was boiled for 5 min, a massive increase of soluble Ure2p was observed (Fig. 5B). Interestingly the presence of urea only slightly changes Ure2p solubility under these conditions. On one hand, urea does not provoke a significant increase in the capacity of Ure2p amyloid fibrils to enter in the polyacrylamide gel (Fig. 5B) but on the other hand, under the same conditions, provokes a 5–10-fold increase in the solubility of aggregated Ure2p in [URE3] cells (Fig. 1A). This suggests that either the amyloids...
produced in vitro are different from in vivo aggregates or that amyloids interaction with proteins from yeast crude extract change their sensitivity toward SDS. We therefore tested whether incubation of amyloids with a yeast extract changed their properties. Recombinant amyloid fibrils were incubated in a crude extract for 5 min prior to addition to sample buffer. Samples were analyzed (after incubation at room temperature or boiling and without adding urea) on a Western blot with antibodies against full-length Ure2p.

Fig. 5. Identification of amyloid fibers. A, the formation of amyloid fibrils from soluble Ure2p was checked by negative staining electron microscopy. B, the same sample was analyzed by SDS-PAGE with Coomassie staining after boiling or not boiling and adding or not adding urea. C, fibrils were incubated 5 min in a crude extract before the addition of the sample buffer. Samples were analyzed (after incubation at room temperature or boiling with or without urea) on a Western blot with antibodies against full-length Ure2p.

Full-length soluble Ure2p is completely degraded with 0.25 μg/ml of proteinase K (Fig. 6A, left). Digestion with 0.05 μg/ml of proteinase K gave rise to eight fragments with molecular masses around 30, 35, 32, 30, 27, 25, 20, and 6 kDa. The 39-, 35-, 32-, and 27-kDa bands were not detected with higher proteinase K concentrations. The fuzzy 30-kDa band was observed with a proteinase K concentration ranging from 0.05 to 0.5 μg/ml. The 25- and 20-kDa bands were observed at a proteinase K concentration up to 0.5 μg/ml. A 18-kDa fragment is detected after digestion from 0.25 to 10 μg/ml of proteinase K, and another 17-kDa fragment is detected from 0.5 to 10 μg/ml. A 10-kDa band is then observed between 2 and 10 μg/ml of proteinase K. The 6-kDa band is observed at up to 0.5 μg/ml of proteinase K. Finally, a 5-kDa fragment is observed between 0.25 and 10 μg/ml of proteinase K. Ure2p is completely degraded with proteinase K concentrations of 50 μg/ml or more.

The Ure2p amyloid fibril digestion pattern is very similar to the one observed for soluble Ure2p, except for two differences (Fig. 6A, right). Firstly, the full-length Ure2p is detected with higher proteinase K concentrations than for soluble protein. Indeed the signal is observed up to 0.5 μg/ml of proteinase K.
The second point is an absence of the 39-, 35-, and 27-kDa fragments in amyloid fibrils proteolysis. Other bands are very similar to the ones observed for soluble protein, in terms of size, sensitivity to the same proteinase K concentrations, and relative intensity (Fig. 6A, compare soluble Ure2p and amyloid fibrils gels).

These samples were further on analyzed on a Western blot probed with the antibody raised against full-length Ure2p protein. All fragments previously observed on Coomassie staining were detected, except the 10- and 5-kDa fragments (Fig. 6, A compare with B). An additional fuzzy fragment not stained by Coomassie was detected ~10 kDa in both cases. This species reveals a dramatic change between soluble and amyloid Ure2p. In soluble Ure2p digestion, this fragment could not be detected anymore for a concentration more than 0.05 µg/ml of proteinase K. On the contrary, it exhibits a strong proteolysis resistance, as it is still detected with 50 µg/ml of proteinase K in amyloid fiber digestions. Previous data showed the Ure2p prion-forming domain is highly resistant to proteolysis in amyloid fibrils and that this fragment has a low affinity for Coomassie dye (23). This strongly suggests that this 10-kDa fragment could be the PFD.

Because the 30-kDa fragments are undetectable in [URE3] cells with the anti-C-terminal antibodies, we tested these antibodies on recombinant Ure2p degradation products. Interestingly, these antibodies gave the same proteolysis pattern for antigens detected with 50 µg/ml of proteinase K. On the contrary, it exhibits a strong proteolysis resistance, as it is still detected with 50 µg/ml of proteinase K in amyloid fiber digestions. Previous data showed the Ure2p prion-forming domain is highly resistant to proteolysis in amyloid fibrils and that this fragment has a low affinity for Coomassie dye (23). This strongly suggests that this 10-kDa fragment could be the PFD.

In [URE3] cells, Ure2p was found in the soluble fraction. In-terestingly, the concentration of Ure2p in [URE3] yeast cells can not be functional anymore. This loss of function could be due either to steric constraints or to a conformational change of the globular domain.

The [URE3] aggregates are resistant to boiling in standard SDS buffer. On the contrary, amyloid fibers assembled in vitro are efficiently denatured by this treatment and do not require urea to be readily detected. This is also the case if fibers are pre-incubated with a yeast crude extract before SDS treatment. This suggests that Ure2p aggregates formed in vitro may be different from in vitro generated amyloid fibers. We used limited proteolysis to study these two types of aggregates.

Limited proteolysis is a powerful tool to investigate protein conformation, as accessible regions are preferentially digested. We compared the digestion pattern of the Ure2p protein from wild-type and [URE3] strains to investigate the conformational changes between the cellular and the prion isoforms. As previously observed, the full-length Ure2p protein was more resistant to proteolysis in [URE3] than in wild-type cells (28, 35, 36). The digestion of the Ure2p cellular isoform resulted in a fuzzy 30-kDa band. It is recognized by an antibody raised against a peptide located 8 kDa upstream of the C-terminal end of the protein. As Ure2p is composed of a globular 30-kDa C-terminal domain (37, 38) and a 10-kDa N-terminal PFD that is unstructured (22), we expected that this unstructured domain would be more sensitive to protease K. Therefore, the 30-kDa fragment detected in our experiment may be the Ure2p functional domain. This hypothesis is supported by a mass spectrometry analyses of recombinant Ure2p digested by proteinase K, because a 30-kDa fragment was identified as the globular domain of Ure2p (39).

Ure2p [URE3] proteolysis gave a distinct pattern. We observed three bands in the range of 26–30 kDa. Our proteolysis pattern is also found in other yeast strains and also a different prion strain (28), indicating that this property is not restricted to our yeast background but is rather a general feature of [URE3]. None of these three bands was detected with the C-terminal antibody. When using the antibodies raised against the full-length Ure2p, the signal intensity of the 30-kDa fragment reaches the same level as undigested Ure2p. As we clearly detected the full-length protein but none of proteolysed fragments with the anti-C-terminal antibodies, these fragments do not include the C-terminal end.

The fragments size is consistent with a first cleavage site located 10 kDa upstream from the C-terminal end of Ure2p and two additional sites separated by 2 kDa. These three fragments are very resistant to proteolysis, as they are detected with up to 50 µg/ml of proteinase K. Ure2p aggregation in [URE3] cells therefore confers an increased resistance to proteolysis. This has been clearly demonstrated in vitro that the resistant part of Ure2p structured into amyloid fibrils is the first 70 amino acids (23). As the biochemical conditions used during proteinase K treatment and Western blot analysis can influence the results, we then wanted to compare the behavior of the four species, recombinant soluble Ure2p, recombinant amyloid Ure2p, cellular Ure2p, and Ure2p""
Only a few differences were observed between the proteolysis patterns of recombinant soluble Ure2p and amyloid fibrils. The first difference is that full-length Ure2p is more protease-resistant when organized in amyloid fibrils. The second is that the 35- and 39-kDa fragments detected in soluble Ure2p digestion are not observed for fibrils. Bouisset et al. (39) have already shown that these fragments corresponded to a proteolysis at the N terminus of the PFD. This indicated that the cleavage sites in the N terminus were less exposed to the solvent in fibrils. The main difference is the presence of a 10-kDa fragment showing a strongly increased resistance to proteinase K in fibrils. This fragment was not stained by Coomassie dye. Previous work from Baxa et al. (23), including mass spectrometry, strongly supported the hypothesis that this fragment may be the PFD. Therefore, the functional domain seems to have the same conformation in fibrils as in soluble protein. This is consistent with the model proposed by Baxa et al. (23) concerning Ure2p fibrillation into an amyloid structure. In this model the PFD forms the backbone of the fibrils, surrounded by the functional domain in a largely native conformation (23).

The comparison of soluble and fibrillar Ure2p can not explain the differences found when crude extracts from wild-type or [URE3] cells are compared by the same approach. In [URE3] crude extracts, the first two-thirds of the protein including the PFD and half of the functional domain of Ure2p exhibit a strong resistance to proteinase K. Ure2p fragments that were obtained by [URE3] crude extract proteolysis are only detected after the addition of urea (data not shown). It means that the peptides are still interacting among themselves. On the other hand, Ure2p amyloid fibrils may be analyzed after proteinase K treatment without the addition of urea. This suggests that Ure2p does not form in vivo the amyloid structures observed in vitro. The difference of behavior between amyloids and [URE3] aggregates could result from the presence of specific partners that would partially cover Ure2p in [URE3] yeast cells. However, the incubation of amyloid fibrils in a yeast crude extract did not change their solubility nor their digestion pattern. This result does not definitively rule out this hypothesis, because such hypothetical partners may interact with Ure2p only during amyloid formation and partially cover Ure2p in [URE3] yeast cells. If this partner exists, it should recognize specifically Ure2p in the aggregated but not the soluble state. This hypothesis is again difficult to combine with the amyloid hypothesis, because in vitro, the polymerization of Ure2p in a filament does not lead to apparent structural changes in the globular part of Ure2p.

Alternatively, this pattern may indicate that not only the PFD, but also part of the functional domain could be the core of the aggregates formed in vivo. This organization implies that the functional domain of Ure2p undergoes a conformational change between the soluble cellular and the aggregated prion isoform. This hypothesis points the fact that the biochemical nature of the aggregates found in the [URE3] yeast cells remains enigmatic.