

## Protein folding and cutaneous diseases

Michelle Gralle Botelho, MD, PhD, and Omar Lupi, MD, PhD

From the Department of Molecular Biology, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany; Department of Dermatology, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), and Post-Graduate Course of Dermatology, Instituto de Dermatologia Rubem David Azulay and Policlínica Geral do Rio de Janeiro, Rio de Janeiro, Brazil

### Correspondence

Michelle Gralle Botelho, MD, PhD  
Department of Molecular Biology  
MPI for Biophysical Chemistry  
37077 Göttingen  
Germany  
E-mail: mgralle1@gwdg.de

### Introduction

Disease processes have different pathogenetic mechanisms, such as infections, nutritional, immunologic and genetic disorders. Specifically, genetic disorders involve mutations in the sequence of a protein, which may lead to its malfunction or complete absence. On the other hand, a malfunctioning protein can cause a dermatologic disease, even though a genetic alteration in its sequence cannot be found. One possible explanation is that certain modifications in the protein structure occur during its lifespan, inducing malfunction and disease. For the majority of these so-called conformational diseases, the exact mechanism that alters protein structure is not known, but insights from general protein folding and misfolding processes will surely help to understand the pathogenesis of such dermatological diseases, and to discover efficient therapeutic methods.

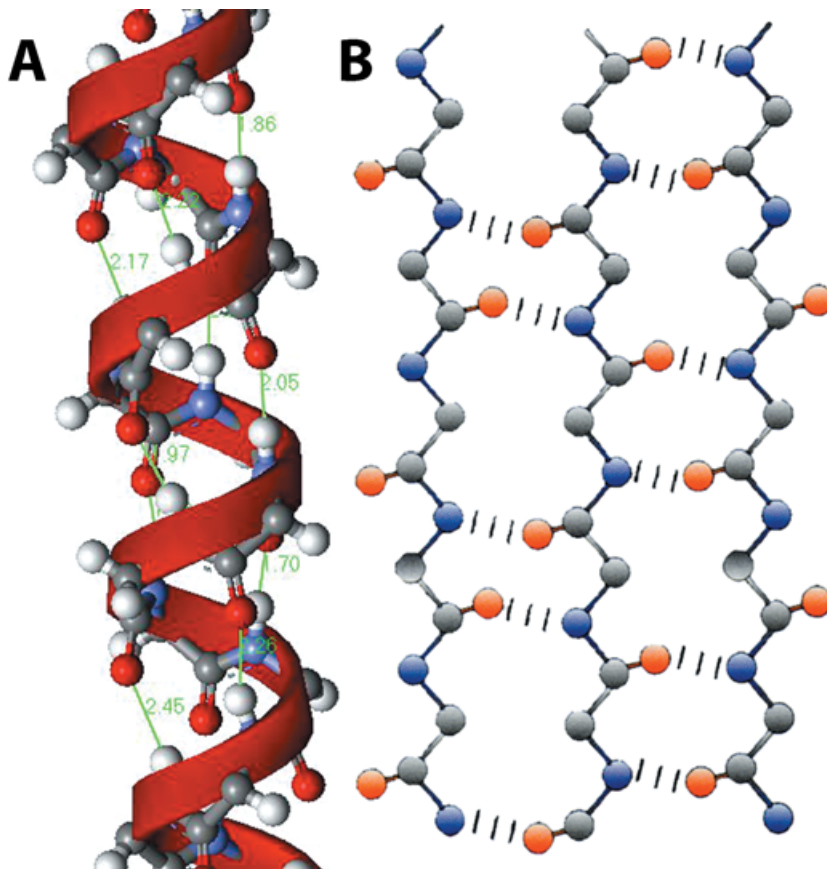
### Protein structure

For a long time it has been known that proteins are a major constituent of cells. In the beginning of the twentieth century, it was shown that some proteins have specific biological functions (e.g. catalytic activity, transport, storage among others). In 1951, Linus Pauling and Robert Corey proposed the first model for local structures of proteins. Twelve years later, Max Perutz showed the first atomic resolution model of a protein. That was a great step towards understanding how a protein functions, and it proved that proteins have a defined conformation, differing from the former notion that proteins were amorphous colloid-like masses.

The primary structure is the linear sequence of amino acids that comprise a protein (its polypeptide chain) as it is encoded by genetic information, and produced by the ribosome. The secondary structure represents the first spontaneous step of the normal folding pathway of the polypeptide chain, and follows certain typical models that Linus Pauling called  $\alpha$ -helices and  $\beta$ -sheets (Fig. 1a,b, respectively). These two structures are present in almost all globular proteins. Globular proteins are composed of a defined number and sequence of these elements of secondary structure that form and organize the whole molecule: the exact spatial arrangement of the atoms in the protein is called its tertiary structure. A drop-like organization, where the hydrophobic amino acids stay in the center, far away from the solvent, and the hydrophilic residues are on the surface, is characteristic of the tertiary structure of globular proteins. In addition to its occurrence in this subtype of proteins, a similar arrangement is also found in the fibrous proteins of our organism. The most common and well-characterized fibrous proteins in the human body are keratin, collagen and elastin. They have simple structures, composed mainly or exclusively of helical elements, which give these kind of proteins a high stability, and that is the reason why these proteins have protective, connective, and supportive roles in living organisms.

### Protein folding

The effectiveness of the normal folding pathway is critical to the determination of the protein function, a fact that can be easily observed with hemoglobin; many diseases are



**Figure 1** Schematic representation of the two major protein secondary structures: alpha helix (a) and a  $\beta$ -sheet (b). The red circles represent the oxygen molecules, the gray circles represent the carbon, the blue the nitrogen groups, and the white represent the hydrogen. On (a) the green lines and numbers show the distances between the molecules (figure reproduced from URL: [http://pharm1.pharmazie.uni-greifswald.de/bednarski\\_web/web/data/personen/Lectures/PMC/Wechselwirkungen/Alpha-helix.jpg](http://pharm1.pharmazie.uni-greifswald.de/bednarski_web/web/data/personen/Lectures/PMC/Wechselwirkungen/Alpha-helix.jpg)). On (B) the dashed lines represent the hydrogen bound between the  $\beta$ -sheets, and the hydrogen molecules and other lateral groups were not shown to simplify. (published with permission from authors of Ref 56)

related to single or multiple mutations on the DNA sequence and will cause diseases because the mutation could change the final protein conformation, just as in sickle cell anemia, thalassemias and other hemoglobin disorders. Another possibility that is now being studied is that even without any DNA mutation some protein conformations could be modified with severe clinical consequences.

Christian Anfinsen, in 1973, proposed that all the information required for the generation of the native three-dimensional conformation of a protein is contained in its amino acid sequence.<sup>1</sup> The big question of folding is to understand how a polypeptide chain can fold rapidly enough to be of use for biological processes. It is clear that a random search of all possible conformations until the best one has been found would not be possible, because this process would take an astronomical amount of time to complete (approximately 20 billion years – Cyrus Levinthal's calculation).<sup>2</sup> Even today this question is not completely solved, but some findings have brought new insights to it. For example, it has been shown that some proteins, called chaperones, have the function to help bigger proteins to fold properly.<sup>3,4</sup> But how do the chaperones fold in the first place? The discovery of intermediates in the folding pathway has also brought new light to the folding question.

Previously in protein folding studies, small proteins were generally considered to fold via a two-state transition involv-

ing a totally unfolded state, without any structure, that progresses directly to a completely folded structure.<sup>5</sup> However, diverse studies showed that many proteins fold through many sequential steps to the final completely folded state: a completely unfolded protein first acquires its secondary structure, then proceeds to the formation of an intermediate with secondary structure, but without a stable tertiary structure, and finally forms a native folded protein.<sup>6</sup> The finding of protein intermediates in the normal folding process led to the important discovery that the same protein can have different stable states, which may participate with different functions in the cell.<sup>7</sup> What seemed at first to be an academic question was proven to be medically important when it was discovered that errors in this folding pathway can lead to many different diseases.

### Misfolding of proteins

Misfolding represents a mistake in the folding pathway that leads to the formation of a protein with a stable final structure, but very different from its native one. Misfolded proteins occur probably all the time in the cell, when a protein is produced in the cytoplasm or in the endoplasmic reticulum, or when an already folded protein suffers a temporary loss of structure in the cell. They can also arise when a mutation in one of the

amino acids of a protein's sequence alters the folding process. These proteins do not normally accumulate and provoke diseases because the cell has an efficient mechanism to detect and destroy these misfolded proteins (through proteasomal degradation).

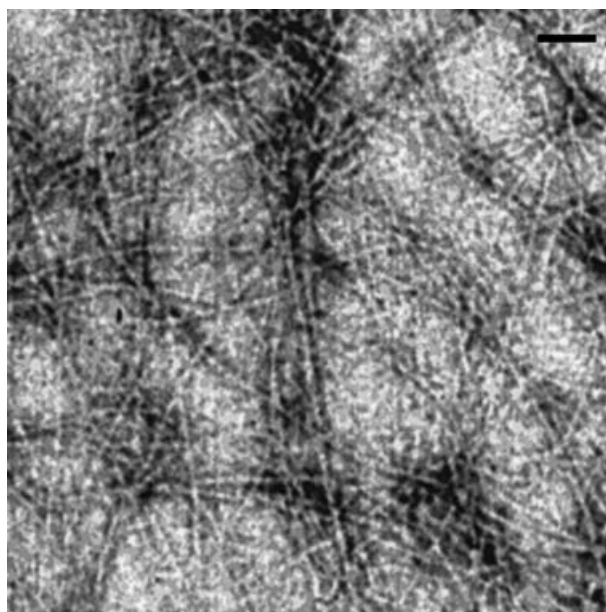
The biological functions of proteins are directly dependent on the acquisition of their precise three-dimensional structures. Failure of the intracellular folding mechanism or of the quality control machinery that detects and eliminates misfolded or nonfolded proteins<sup>8</sup> may lead to major pathological conditions collectively described as conformational diseases.<sup>9</sup> This failure in the control machinery of the cells may represent a sign of cell aging and may explain why some of the conformational diseases manifest themselves in aged patients.

The misfolded proteins that are not destroyed by the cell tend to accumulate inside or outside the cell, and they can associate to form large aggregates of proteins. Actually, aggregation is the most common destiny of a misfolded protein, if it is not destroyed.<sup>8</sup> Until some years ago, it was believed that these aggregates were extremely toxic for cells and were the main cause of cell death, for example, the deposition of  $\beta$ -amyloid fibrils in Alzheimer's disease. Today, an increasing quantity of new data suggests that the first stages of aggregation, which are called protofibrils or prefibrillar aggregates, are much more toxic and dangerous than the mature fibrils.<sup>10</sup> For example, this may explain the lack of existence of a direct correlation between the density of fibrillar plaques in the brains of patients with Alzheimer's disease (often proposed to cause dementia) and the severity of clinical symptoms.<sup>11</sup> Therefore, a new question appeared: are the large fibrillar aggregates the major pathologic event that leads to cell death and tissue dysfunction or are they the last option for cells to get rid of the misfolded protein that accumulates in their inside? The answer to this question is of extreme importance, because any therapeutic approach should be directed against the core problem.

The exact pathologic mechanisms that lead to cell death have not yet been completely understood. Some evidence showed that the prefibrillar aggregates have some exposed amino acid regions that can mimic regions of the surface of the native protein. Therefore, these regions are likely to be able to interact inappropriately with the binding partners or receptors of the native proteins.<sup>12</sup> Indeed, it has been shown that some prefibrillar aggregates can interact with cell membranes, possibly destabilizing them, and leading to cell death.<sup>13</sup>

Furthermore, the protein misfolding process might have a direct toxic effect on the cell, and/or it might provoke the loss of a vital function of the cell due to the absence of the correctly folded protein: in both cases it leads to cell death. This theory is called "gain of function or loss of function" and explains the pathologic mechanism of the conformational diseases.

The amyloidosis and the prion diseases are the most well-known and important conformational diseases.



**Figure 2** Electron microscopy of lysozyme fibrils. The fibrils were produced at pH 2, 65 °C. Scale bar: 150 nm. Taken with permission from authors of Ref 15

## Amyloidosis

The first person to detect the protein aggregates mentioned above and to associate them with disease was Virchow in 1854. After reacting, the fibril aggregates were visible on hepatic tissue with iodine and sulfuric acid. Since that is the same reaction used to stain starch (*amylum*), Virchow gave the name amyloid to this type of aggregate. More than 70 years later, Divry and Florkin stained the amyloid aggregates with the dye Congo Red and recognized that they showed an apple-green birefringence when analyzed under polarized light (Fig. 2a).<sup>14</sup> To the present day, this has remained the most widely used method to diagnose an amyloid disease. Later, electron microscopic analysis showed that all such aggregates exhibit a similar fibrillar structure, with bundles of straight, rigid fibrils ranging in width from 60 to 130 Å and in length from 1000 to 16,000 Å (Fig. 2b).<sup>15</sup>

The amyloid diseases comprise important pathologies, including Alzheimer's and Parkinson's diseases, type II diabetes mellitus and various forms of systemic and cutaneous amyloidosis (Table 1). The prion diseases are not universally considered amyloidosis because it is not clear if the amyloid aggregates present in the prion diseases are directly implicated with the neurodegeneration or only represent an epiphenomenon in the disease (see also below); still, the prion diseases were included in this review because of the possibility of a cutaneous infection route.

Some of the amyloidoses are genetically inherited, and point mutations of normally occurring proteins have been described

**Table 1** Most well-known amyloidosis

Disease	Causative protein	Affected sites
Alzheimer's disease	$\beta$ -amyloid peptide	Central nervous system (CNS)
Parkinson's disease	$\alpha$ -synuclein	CNS, dopaminergic centers
Familial amyloid polyneuropathy	Transthyretin	Heart, liver, peripheral nerves
Huntington's disease	Huntingtin	CNS
Creutzfeldt-Jakob's disease	Prion protein	CNS
Amyloidosis related to hemodialysis	$\beta$ 2-microglobulin	Kidneys
Type II diabetes mellitus	Amylin	Pancreatic islets
Systemic primary amyloidosis	AL amyloid derived from the immunoglobulin light chain	Many organs: skin, heart, liver, kidneys
Secondary amyloidosis	Serum Amyloid Protein (SAP)	Many organs: skin, heart, liver, kidneys
Primary localized cutaneous amyloidosis	Immunoglobulin light chains (nodular form) Keratin??	Skin

for many familiar forms of amyloidosis. These point mutations may not always alter the protein structure, but somehow favor the aggregation of the protein, and leads to its accumulation in the tissue and disease. Some examples of familial forms of amyloidosis are familial amyloidotic polyneuropathy I, or Corino de Andrade's disease, caused by transthyretin mutants;<sup>16</sup> familial forms of Alzheimer's disease, caused by  $\beta$ -amyloid peptide,<sup>17</sup> and Huntington's disease, caused by huntingtin.<sup>18</sup> Since these mutations favor the aggregation of the respective proteins, the disease symptoms are seen rather early in the afflicted members of the families and do not reflect simply a cell-aging process. Recently, a familial form of primary cutaneous amyloidosis has also been described and linked to alterations on chromosome 5.<sup>19</sup>

The most peculiar aspect of the amyloid diseases is the fact that many proteins associated with this condition do not have any similarity in size, sequence or native structure. However, they are all able to form amyloid fibrillar aggregates, which when closely studied, showed a marked similarity in morphology and internal structure. In general, the amyloid fibers are insoluble, resistant to proteolysis and show an extremely high content of  $\beta$ -sheet (Fig. 1a).<sup>12</sup> Another interesting aspect of the amyloidogenic process is that apparently, under the right conditions, any protein can form amyloid aggregates, even disease-unrelated proteins.<sup>20</sup> These required conditions can be, for example, low pH, lack of specific ligands, high temperature, presence of salts or cosolvents which disrupt the protein's native structure; and in many cases the fibrils formed do not represent a physiological reality. However, the possibility to study such artificial amyloid fibrils was helpful in understanding the amyloidogenic process better, and the mere existence of these fibrils proved that the ability to form amyloid fibrils is a generic property of peptides and proteins. It seems that the disease-causing mutations in familiar amyloidosis merely facilitate and accelerate the formation of such fibrils.

The exact process by which a soluble protein forms insoluble aggregates is not totally defined, although the understanding of the mechanism by which a protein assumes an amyloido-

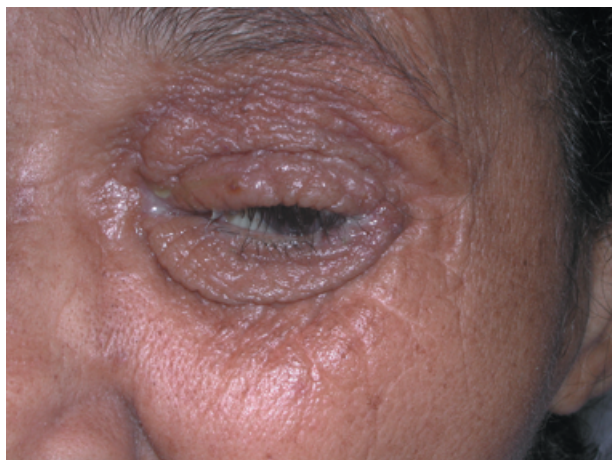
genic conformation is crucial to the development of future therapeutic approaches.

Shown below is an overview of the systemic and localized amyloidosis, including their cutaneous symptoms. Since it is not the objective of this review to give a profound analysis of these forms of amyloidosis, only the more important features are mentioned.

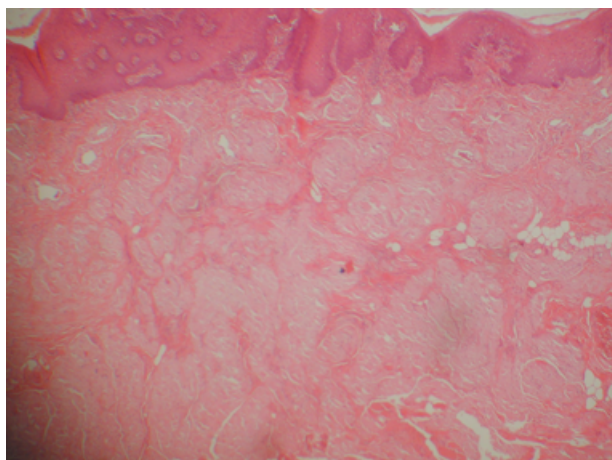
### Systemic amyloidosis

The most frequent types of systemic amyloidosis are the AL (primary) and the AA (secondary) types (for a detailed review of systemic amyloidosis, see<sup>21</sup>). AL amyloidosis is caused by the accumulation of the AL protein that is derived from the light chain of immunoglobulin, more precisely from the  $\lambda$ -region in 75% of the cases, and from the  $\kappa$ -region in the remaining.<sup>22</sup> This accumulation causes deposition of the protein aggregates in different tissues, and finally, organ dysfunction. The AL amyloidosis can be associated with multiple myeloma and Waldenstrom macroglobulinemia. AL amyloidosis can affect only one organ, or have a multiorgan involvement, and the main clinical presentations are: renal involvement with nephrotic syndrome, restrictive cardiomyopathy, peripheral neuropathy (frequently presenting as carpal tunnel syndrome), hepatomegaly with or without splenomegaly, macroglossia and many unspecific cutaneous signs. The most common cutaneous presentations include a waxy thickening of the skin, ecchymoses, purpura (characteristically after Valsalva maneuver) and subcutaneous nodules and plaques (Fig. 3). The ecchymoses and purpura are due to the perivascular infiltration of the amyloid deposits (Fig. 4), which cause a local inflammatory reaction, and are found more frequently in flexural regions, such as the eyelids, nasolabial folds and neck, besides the mouth. The nails can be also affected, and brittleness, crumbling, subungual striations and partial onychia may be present.

AA amyloidosis is a secondary form of amyloidosis that may complicate chronic diseases in which there is chronic or recurrent inflammation, such as rheumatoid arthritis



**Figure 3** Systemic Amyloidosis. Eyelid infiltration with multiple papules



**Figure 4** Systemic Amyloidosis. Deposition of abundant amorphous material forming large nodules within the dermis and is accentuated around vessels (H&E,  $\times 250$ ). (Courtesy of Bernard Kac, MD)

or spondyloarthropathy; chronic infections; or periodic fever syndromes. The fibrils are composed of fragments of the acute phase reactant serum amyloid A. Just as in AL amyloidosis, the most affected organs are kidneys, liver, and heart.

The diagnosis can be suspected by the history and findings in the physical exam, and by the presence of the light chains in a urine sample. However, the diagnosis can only be confirmed by tissue biopsy and the demonstration of amyloid deposits (Fig. 4). There is no optimal biopsy site: biopsy can be directed at dysfunctional organs, such as kidneys or nerves, or at other sites, such as subcutaneous fat, salivary glands or rectal mucosa. A new diagnostic method available in some special-

ized centers is mass-spectroscopy of the paraffin embedded specimen, which allows a precise identification of the protein involved in the amyloid formation.<sup>23</sup>

In conclusion, both forms of systemic amyloidosis have in common a high expression rate of a specific protein, immunoglobulin light chain in the AL amyloidosis, and serum amyloid A in the AA form. But how an excess of these proteins leads to their aggregation and the formation of amyloid fibrils is still not clear.

### Localized amyloidosis

Different from the systemic AL amyloidosis, there are some patients who present localized deposition of the immunoglobulin light chains; this will cause damage to the affected site, usually upper respiratory tract (nasopharynx, lips), colon, orbit, skin and nails.<sup>24,25</sup>

The primary localized cutaneous amyloidosis comprises the macular, papular (or lichen amyloidosis) and the nodular forms. The nodular form can be considered a localized plasmacytoma because a high production of immunoglobulin light chains by clonally expanded plasma cells is observed, just as in the systemic AL amyloidosis, but locally.<sup>26</sup> On the other hand, the other forms of localized cutaneous amyloidosis are not associated with immunoglobulin production, and their pathogenesis is not completely clear. It is believed that a focal epidermal damage is involved, with a local degeneration of keratinocytes, followed by apoptosis and conversion of filamentous masses (colloid bodies) into amyloid material in the papillary dermis.<sup>27</sup> Indeed there are evidences that the keratinocytes are involved with the fibrils formation<sup>28</sup> with the presence of keratins in the fibrils.<sup>29</sup> The exact mechanisms which cause the epidermal damage and how the amyloid fibrils are formed is still unknown. Some believe that chronic pruritus might lead to keratinocyte degeneration and initiation of the amyloid formation.<sup>30,31</sup>

The clinical presentation of nodular amyloidosis is the presence of one or more nodular lesions in the limbs, trunk, face, or genitalia. The nodules' size can vary from few millimeters to several centimeters.<sup>32</sup> It is a benign lesion, but in a few patients it can evolve to systemic paraproteinemia and systemic amyloidosis.<sup>33,34</sup> A possible treatment is the excision of the lesion.<sup>35</sup>

In the lichen amyloidosis form, the individual lesions are smooth or hyperkeratotic papules which may coalesce to form pruritic plaques, presented principally on the shins. Finally, the macular form is characterized by poorly delineated, hyperpigmented patches with a chronic evolution and affects the upper back, limbs and occasionally chest and buttocks. This form can be pruriginous. In both cases the diagnosis is made through the biopsy of the affected region with the demonstration of the amyloid deposits in the papillary dermis. There is no effective treatment for these two forms of

cutaneous amyloidosis; topical steroids combined with antihistamines can be used to alleviate the pruritus in some cases. Calcipotriol, tretinoin or acitretin therapy have also been tested.<sup>36,37</sup> The use of topical dimethylsulfoxide (DMSO), an anti-inflammatory compound that readily penetrates the skin and is sometimes useful to relieve pain and itch in amyloidosis, is still controversial.<sup>38,39</sup>

### Prion infections

The exact nature of transmissible spongiform encephalopathies (TSE) perplexed researchers for decades, until Prusiner identified and defined prion infections in 1982.<sup>40</sup> Prusiner classified a prion as an infectious particle composed of a protein (PrP) that causes fatal neurodegenerative disorders.<sup>40</sup> According to Collinge, prions are infectious agents by biological and medical criteria, but with fairly unique properties that differ from those of conventional microbes.<sup>13</sup>

Prions cause a variety of degenerative neurologic diseases that can be infectious, inherited, or sporadic in origin.<sup>13</sup> The cause of the sporadic forms is unknown; inherited forms are caused by up to 20 different mutations of the human PrP gene; and the infectious forms are transmitted through contact with or consumption of previously infected tissues.<sup>13</sup>

Well known human prion diseases include Creutzfeldt-Jakob disease (CJD), kuru, German–Straussler–Scheinker syndrome (GSS), and fatal familial insomnia (FFI).<sup>13</sup> Other well-known prion diseases that affect animals include scrapie (sheep and goats), bovine spongiform encephalopathy (BSE or mad cow disease), transmissible mink encephalopathy, chronic wasting disease (CWD) in cervids, feline spongiform encephalopathy, and exotic ungulate encephalopathy.<sup>13,41</sup> BSE has been described as affecting humans through ingestion of contaminated meat, causing variant CJD (vCJD) in Europe.<sup>41</sup>

PrP<sup>C</sup> is the normal, cellular prion protein, and in affected organisms it is converted into PrP<sup>Sc</sup> (from scrapie). The normal PrP<sup>C</sup> consists mainly of alpha helices, but the converted PrP<sup>Sc</sup> is predominantly formed by beta-sheets.<sup>13,40</sup> The initially highly controversial, but today widely accepted mechanism of replication involves the conversion of cellular proteins to the pathological conformation without involvement of nucleic acid templates, and such a conversion possibly opens the way for other strain-specific post-translational modifications of the prion protein.<sup>13,40</sup> This change initiates a chain reaction, and newly converted prions convert other proteins, which they probably come into contact with on the cell membrane.<sup>40</sup> The PrP<sup>Sc</sup> accumulates in lysosomes and eventually fills the lysosomes until they burst, releasing the prions to attack other cells.<sup>13</sup>

The skin and mucous membranes are a potential target for prion infections, since keratinocytes and lymphocytes are susceptible to the abnormal infective isoform of the prion

protein. Iatrogenic transmission of Creutzfeldt-Jakob disease was also recognized after corneal transplants in humans and scrapie was successfully transmitted to mice after ocular instillation of infected brain tissue, confirming that these new routes could also be important in prion infections.<sup>41,42</sup>

Although bovine collagen has long been recognized as a safe and biocompatible material, physicians should be aware of the potential for prion transmission when using materials from bovine origin. Bovine collagen has been used *in natura*, as a filling material, and is potentially associated with prion rod transmission since BSE reached epidemic proportions for almost 8 years in Europe.<sup>41,42</sup>

Another important point is that the use of sterile technique in cell cultivation is absolutely essential. Sterilization by autoclaving relies on steam pressure and high heat. Many solutions, including standard cell culture medium cannot be sterilized by this procedure. Laboratories add newborn calf serum (NCS) to most of the culture medium as a supplement. Prions have a theoretical risk to be transmitted to the cell culture medium by previous infected NCS. Some cell lines are very susceptible to this infection, such as fibroblasts, neuronal cells and hypothalamic cell line. After transmission to these cells, all the resultant products also will be infected with prions.<sup>41-43</sup>

Some ectoparasites have been proven to harbor prion rods in laboratory experiments.<sup>42-43</sup> Prion rods were identified in both fly larvae and pupae; adult flies are also able to express prion proteins.<sup>43</sup> The most common causes of myiasis in cattle and sheep, animals closely related with previous prion infections, are *Hypoderma bovis* and *Oestrus ovis*, respectively.<sup>42,43</sup> Both species of flies present a life cycle very different from human myiasis, since they present a long contact with neurologic structures, which are potentially rich in prion rods. Ophthalmomyiasis in humans is commonly caused by both species of fly larvae worldwide, providing an almost direct contact with the central nervous system. The high expression of the prion protein on the skin and mucosa and the huge amount of inflammatory response to the larvae could readily increase the efficiency of transmission of prions.<sup>41-43</sup>

### Prions and amyloids: the concept of transmissible cerebral amyloidosis

The amyloid plaque has long been recognized as a hallmark of the neuropathology of some TSEs, especially GSS and kuru.<sup>44</sup> Three decades later, it was established that the amyloid plaque of TSE was mostly composed of misfolded PrP. As a result, Gajdusek suggested calling PrP 27-30, the shortest truncated form of PrP which still retains infectious properties, a “scrapie amyloid”.<sup>45</sup>

Fibrillar structures isolated from TSE-affected brains (prion rods) are morphologically very similar to but distinguishable from other amyloid fibrils when visualized by negative-staining electron microscopy.<sup>46</sup> Direct evidence for a cross pleated secondary structure of PrP<sup>Sc</sup> was obtained within the last few

years by infrared spectroscopy, which correlates the infrared spectrum with the secondary structure of proteins. Caughey *et al.* showed that PrP 27–30 contains a high proportion of pleated sheet.<sup>47</sup> Denaturation by either SDS or high pH reduces both scrapie infectivity and pleated content.<sup>48</sup>

PrP fragments are able to form either helices or pleated sheets and conversion from helix into a sheet may underlie the formation of prions. Indeed, using hamster recombinant protein, Mehlhorn *et al.* demonstrated that the same peptide may form stable helix or sheets with several intermediates.<sup>49</sup> All these data point to an inherent propensity of PrP to exist in different conformations.

Riek *et al.* described the secondary structure of mouse PrP in solution to consist of three helices and 2 antiparallel strands at the globular C-terminus.<sup>50</sup> A comparison between human, mouse and bovine PrP demonstrated that human and bovine PrPs exhibit virtually identical conformations,<sup>51</sup> and because species susceptibility is strongly influenced by PrP conformational similarity, humans are very susceptible for BSE infection.

As detailed above, it is now widely accepted that structurally diverse proteins can misfold and cause so-called “conformational diseases” including systemic and cutaneous amyloidosis, Alzheimer’s disease and Parkinson’s disease. All these diseases are characterized by amyloid plaques that may be causally connected to the pathological process. In the prion diseases GSS, vCJD and in some murine scrapie models of disease, “classical” or multicentric (in GSS) amyloid plaques are frequent, but they are often absent in human TSE cases,<sup>52</sup> in BSE and most ovine scrapie.

All these neurodegenerative diseases are “infectious” in the sense that misfolded sheeted conformers formed in a nucleation process in which preformed metastable oligomer acts as a seed to convert a normal into an abnormal protein.<sup>53–55</sup> However, in none but TSE has infectivity in a microbiological sense (from one organism to another) ever been observed, and even in TSE the formation of misfolded protein is not necessarily accompanied by the generation of infectivity *de novo*. Lundmark *et al.*<sup>54,55</sup> recently described the transmissibility of systemic amyloidosis by a prion-like mechanism. Mice were orally infected by a homogenate of amyloid extracted from the liver of mice previously exposed to silver nitrate, a substance that is classically related to the induction of AA amyloidosis,<sup>54</sup> or by naturally occurring amyloid fibrils.<sup>55,56</sup> The transmission occurred in a very effective way and suggested that at least some forms of amyloidosis may be transmissible diseases, akin to the prion-associated disorders.

## Conclusion

The aim of this review has been to give insights about normal folding, and to introduce the problems that occur when a protein follows a wrong pathway and ends in a wrong final conformation. This new class of diseases called conformational diseases

is characterized by the formation of misfolded proteins that have the ability to aggregate into fibrils and cause tissue/organ dysfunction and disease. The exact mechanisms that lead to this misfolding and aggregation are not yet known for all diseases of this class.

It seems critical to recognize both prion diseases and the different forms of amyloidosis, such as systemic and cutaneous amyloidosis, as different examples of conformational diseases which depend on the conversion of normal proteins into an “amyloidogenic”, mostly  $\beta$ -sheet structure liable to aggregation. In this interpretation, prion diseases distinguish themselves by their cerebral localization, and their transmission between organisms has been proved beyond doubt; a cutaneous transmission seems possible. Systemic amyloidosis, on the other hand, afflicts several organs of the body, among them the skin, and its transmission between organisms has yet to be shown to occur outside the laboratory. The same is true for cutaneous amyloidosis.

The precise identification of the conformational diseases and the understanding of their pathogenesis are fundamental for the development of new and effective treatment.

## References

- 1 Anfinsen CB, Haber E, Sela M, *et al.* Kinetics of formation of native ribonuclease during oxidation of reduced polypeptide chain. *Proc Natl Acad Sci USA* 1961; 47: 1309–1314.
- 2 Levinthal C. *Proceedings in Mössbauer Spectroscopy in Biological Systems*. Urbana: University of Illinois Press, 1969: 22–24.
- 3 Hemmingsen SM, Woolford C, Vandervies SM, *et al.* Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* 1988; 333: 330–334.
- 4 Bechtluft P, van Leeuwen RGH, Tyreman M, *et al.* Direct observation of chaperone-induced changes in a protein folding pathway. *Science* 2007; 318: 1458–1461.
- 5 Tanford C. Protein denaturation. *Adv Protein Chem* 1968; 23: 121–282.
- 6 Ikai A, Tanford C. Kinetic evidence for incorrectly folded intermediate states in the refolding of denatured proteins. *Nature* 1971; 230: 100–102.
- 7 True HL, Lindquist SL. A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* 2000; 407: 477–483.
- 8 Bukau B, Weissman J, Horwich A. Molecular chaperones and protein quality control. *Cell* 2006; 125: 443–451.
- 9 Gregersen N, Bross P, Andresen BS, *et al.* The role of chaperone-assisted folding and quality control in inborn errors of metabolism: Protein folding disorders. *J Inherit Metab Dis* 2001; 24: 189–212.
- 10 Lambert MP, Barlow AK, Chromy BA, *et al.* Diffusible, nonfibrillar ligands derived from A $\beta$ 1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA* 1998; 95: 6448–6453.

- 11 Dickson DW, Crystal HA, Bevona C, *et al.* Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiol Aging* 1995; 16: 285–298.
- 12 Bucciantini M, Giannoni E, Chiti F, *et al.* Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* 2002; 4: 507–511.
- 13 Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; 24: 519–550.
- 14 Divry P, Florkin M. The optic properties of amyloid. *C R Seances Soc Biol Fil* 1927; 97: 1808–1810.
- 15 Vieira MN, Figueroa-Villar JD, Meirelles MN, *et al.* Small molecule inhibitors of lysozyme amyloid aggregation. *Cell Biochem Biophys* 2006; 44: 549–553.
- 16 Ando Y, Nakamura M, Araki S. Transthyretin-related familial amyloidotic polyneuropathy. *Arch Neurol* 2005; 62: 1057–1062.
- 17 Goate A, Chartierharlin MC, Mullan M, *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 1991; 349: 704–706.
- 18 Norremolle A, Ries O, Epplen JT, *et al.* Trinucleotide repeat elongation in the Huntingtin gene in Huntington disease patients from 71 Danish families. *Hum Mol Genet* 1993; 2: 1475–1476.
- 19 Lee DD, Lin MW, Chen IC, *et al.* Genome-wide scan identifies a susceptibility locus for familial primary cutaneous amyloidosis on chromosome 5p13.1-q11.2. *Br J Dermatol* 2006; 155: 1201–1208.
- 20 Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J Mol Med* 2003; 81: 678–699.
- 21 Obici L, Perfetti V, Palladini G, *et al.* Clinical aspects of systemic amyloid diseases. *Biochim Biophys Acta* 2005; 1753: 11–22.
- 22 Perfetti V, Casarini S, Palladini G, *et al.* Analysis of V $\lambda$ -J $\lambda$  expression in plasma cells from primary (AL) amyloidosis and normal bone marrow identifies 3r ( $\lambda$  III) as a new amyloid-associated germline gene segment. *Blood* 2002; 100: 948–953.
- 23 Kaplan B, Martin BM, Livneh A, *et al.* Biochemical subtyping of amyloid in formalin-fixed tissue samples confirms and supplements immunohistologic data. *Am J Clin Pathol* 2004; 121: 794–800.
- 24 Guidelines Working Group of UK Myeloma Forum; British Committee for Standards in Haematology, British Society for Haematology. Guidelines on the diagnosis and management of AL amyloidosis. *Br J Haematol* 2004; 125: 681–700.
- 25 Fujita Y, Tsuji-Abe Y, Sato-Matsumura KC, *et al.* Nail dystrophy and blisters as sole manifestations in myeloma-associated amyloidosis. *J Am Acad Dermatol* 2006; 54: 712–714.
- 26 Grunewald K, Sepp N, Weyrer K, *et al.* Gene rearrangement studies in the diagnosis of primary systemic and nodular primary localized cutaneous amyloidosis. *J Invest Dermatol* 1991; 97: 693–696.
- 27 Steciuk A, Domp Martin A, Troussard X, *et al.* Cutaneous amyloidosis and possible association with systemic amyloidosis. *Int J Dermatol* 2002; 41: 127–132.
- 28 Horiguchi Y, Suzuki S, Toda K, *et al.* Three-dimensional cultures of keratinocytes and an application to in vitro amyloid production of cutaneous amyloidosis. *Microsc Res Tech* 1997; 38: 38793.
- 29 Huilgol SC, Ramnarain N, Carrington P, *et al.* Cytokeratins in primary cutaneous amyloidosis. *Australas J Dermatol* 1998; 39: 81–85.
- 30 Wong CK, Lin CS. Friction amyloidosis. *Int J Dermatol* 1988; 27: 302–307.
- 31 Iwasaki J, Aki T, Yoshida Y, *et al.* “Thermie” and friction therapy performed using a special instrument. *J Dermatol* 2007; 34: 486–489.
- 32 Santos-Juanes J, Galache C, Curto JR, *et al.* Nodular primary localized cutaneous amyloidosis. *J Eur Acad Dermatol Venereol* 2004; 18: 224–226.
- 33 Woollons A, Black MM. Nodular localized primary cutaneous amyloidosis: a long-term follow-up study. *Br J Dermatol* 2001; 145: 105–109.
- 34 Kalajian AH, Waldman M, Knable AL. Nodular primary localized cutaneous amyloidosis after trauma: a case report and discussion of the rate of progression to systemic amyloidosis. *J Am Acad Dermatol* 2007; 57: S26–S29.
- 35 Bozиков K, Janezic T. Excision and split thickness skin grafting in the treatment of nodular primary localized cutaneous amyloidosis. *Eur J Dermatol* 2006; 16: 315–316.
- 36 Khoo BP, Tay YK, Goh CL. Calcipotriol ointment vs.  $\beta$ -methasone 17-valerate ointment in the treatment of lichen amyloidosis. *Int J Dermatol* 1999; 38: 539–541.
- 37 Hernandez-Nunes A, Dauden E, de Vega MJM, *et al.* Widespread biphasic amyloidosis: response to acitretin. *Clin Exp Dermatol* 2001; 26: 256–259.
- 38 Ozkaya-Bayazit E, Kavak A, Gungor H, *et al.* Intermittent use of topical dimethyl sulfoxide in macular and papular amyloidosis. *Int J Dermatol* 1998; 37: 949–954.
- 39 Pandhi R, Kaur I, Kumar B. Lack of effect of dimethylsulphoxide in cutaneous amyloidosis. *J Dermatolog Treat* 2002; 13: 11–14.
- 40 Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; 216: 136–144.
- 41 Lupi O. Prions in dermatology. *J Am Acad Dermatol* 2002; 46: 790–793.
- 42 Lupi O. Could ectoparasites act as vectors for prion diseases? *Int J Dermatol* 2003; 42: 425–429.
- 43 Lupi O. Risk analysis of ectoparasites acting as vectors for chronic wasting disease. *Med Hypotheses* 2005; 65: 47–54.
- 44 Klatzo I, Gajdusek DC, Zigas V. Pathology of Kuru. *Laboratory Invest* 1959; 8: 799.
- 45 Gajdusek DC. Transmissible and nontransmissible dementias – Distinction between primary cause and pathogenetic mechanisms in Alzheimers-Disease and aging. *Mt Sinai J Med* 1988; 55: 3–5.

- 46 Prusiner SB, McKinley MP, Bowman KA, *et al.* Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* 1983; 35: 349–358.
- 47 Caughey BW, Dong A, Bhat KS, *et al.* Secondary structure-analysis of the scrapie-associated protein PRP 27–30 in water by infrared-spectroscopy. *Biochemistry* 1991; 30: 7672–7680.
- 48 Brown P, Liberski PP, Wolff A, *et al.* Conservation of infectivity in purified fibrillary extracts of scrapie-infected hamster brain after sequential enzymatic digestion or polyacrylamide-gel electrophoresis. *Proc Natl Acad Sci USA* 1990; 87: 7240–7244.
- 49 Mehlhorn I, Groth D, Stockel J, *et al.* High-level expression and characterization of a purified 142-residue polypeptide of the prion protein. *Biochemistry* 1996; 35: 5528–5537.
- 50 Riek R, Hornemann S, Wider G, *et al.* NMR structure of the mouse prion protein domain PrP (121–231). *Nature* 1996; 382: 180–182.
- 51 Lopez-Garcia F, Zahn R, Riek R, *et al.* NMR structure of the bovine prion protein. *Proc Natl Acad Sci USA* 2000; 97: 8334–8339.
- 52 Budka H, Aguzzi A, Brown P, *et al.* Neuropathological diagnostic-criteria for Creutzfeldt-Jakob-Disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol* 1995; 5: 459–466.
- 53 Lupi O, Peryassu MA. An emerging concept of prion infections as a form of transmissible cerebral amyloidosis. *Prion* 2007; 1: 223–227.
- 54 Lundmark K, Westermark GT, Nystrom S, *et al.* Transmissibility of systemic amyloidosis by a prion-like mechanism. *Proc Natl Acad Sci USA* 2002; 99: 6979–6984.
- 55 Lundmark K, Westermark GT, Olsen A, *et al.* Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: Cross-seeding as a disease mechanism. *Proc Natl Acad Sci USA* 2005; 102: 6098–6102.
- 56 Botelho MG, Azulay DR. Amiloidoses. *Dermatologia*. Rio de Janeiro: Guanabara Koogan, 2006: 456–458.