Abnormal Proteins, Protein-Diseases

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Introduction

This chapter is focused on protein-diseases, exemplified by the well-known Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob disease, and other serious pathological disorders. The chapter first summarizes the most important features and disorders of protein structure, and later describes the genetic and biochemical background, pathophysiology and potential cures of the most prevalent protein diseases.

Misfolded Proteins

Major Features of Protein Structure

Proteins can be described as long chains of their constituent 20 amino acids. A protein of typical length contains 200 to 500 amino acids. These protein chains have to form a unique 3-dimensional pattern. The various pathways leading to this final, native conformation are called together as the protein folding process. Amino acid side chains have special physico-chemical features which are essential for the enzyme activity, binding or complex formation of the hosting protein with other molecules, such as small ligands, proteins, RNA or DNA. The surface of proteins (which includes their binding sites) is defined by the network of interactions between amino acids. The energy which is liberated when the individual interactions between the amino acids are formed helps to overcome the difficulty of making an organized, ordered structure (the loss of entropy) during the folding process. The protein structure is stabilized by hydrophobic, van der Waals, hydrogen-bond and ionic interactions. Protein structure is described by four hierarchical levels. The *primary structure* denotes the amino acid sequence, while the *secondary structure* summarizes the local structural elements. According to the traditional view, α-helices and β-sheets are the most prevalent of the repetitive secondary structures. However, recent data suggest a rather large abundance of polyprolin II-helix as well. The non-repetitive secondary structure elements contain β-turns and coiled (unstructured) conforma-

tions. The *tertiary structure* means the complete three-dimensional structure of a polypeptide chain. The *quaternary structure* refers to the complex structure of a protein containing more than one individual polypeptides or other larger constituents (such as heme, or polysaccharide) besides the polypeptide.

Folding of globular proteins starts with hydrophobic collapse in with the internal, hydrophobic core of the protein is formed in a rapid process. This initial phase is followed by a slow re-annealing of the internal structure of folding intermediates, often called molten globules. In this phase, the proteins still have hydrophobic surfaces, which make them very vulnerable to aggregation. At the closing phase, the individual, high-energy bonds (such as ionic interactions or disulfide-bridges, if any) and high-energy active sites are formed. The folding process is often fast (in the range of milliseconds) in case of small proteins (below 30 kDa), but the final rearrangement of the hydrophobic core may require weeks without any extra help.

The evolution of a stable protein structure was not an easy and straightforward process. During this lengthy procedure, special amino-acid sequences had to be selected which are able to fold into unique, native structures. In principle, an amino acid sequence encodes a specific 3-dimensional structure of a protein. According to this view, a protein chain does not require any help to develop the final, native structure. This is indeed true for the dilute solutions of proteins smaller than 30 kDa. However, the folding of larger proteins, especially at the large protein concentration of 300 to 400 mg/ml inside the cell, is not possible without help. Under such conditions (called molecular crowding), the chance of protein aggregation is extremely increased since the hydrophobic surfaces of the molten globule intermediates have a very high chance of getting close to each other. Aggregation is a great danger since in many cases it irreversibly incapacitates the participating proteins.

Cells have developed two methods to prevent, repair or eliminate protein aggregation. Protein-aggregation is prevented by *molecular chaperones* which transiently bind to native, freshly synthesized proteins, or to misfolded, damaged proteins in the cell. During this process chaperones cover hydrophobic surfaces and prevent their binding to each other, which otherwise leads to aggregation. Most types of cellular stress induce an elevated synthesis of several molecular chaperones; therefore, many major chaperones are called heat shock proteins (e.g. Hsp60, Hsp70 or Hsp90, where the numbers refer to the molecular weight of the protein class in kDa). Heat shock pro-

teins are present in unstressed, normal cells since they are needed in various, 'normal' conformational rearrangements, such as protein complex formation or protein transport across the membranes. Some initial protein aggregates can be desaggregated by the Hsp40/Hsp104 or Hsp40/Hsp70 chaperone machines.

In eukaryotic cells, including human cells, the elimination of misfolded and aggregated proteins is mostly processed by a protein complex called proteasome. This mechanism was discovered by Aaron Ciechanover, Irwin Rose and the Hungarian-born Avram Hershko, who received a Nobel Prize for their seminal findings. The first step is the recognition of misfolded proteins by one type of the *ubiquitin-conjugating enzyme* family, which covalently labels misfolded proteins with an oligomer (n > 4, smaller oligomers serve a signalling function) called ubiquitin of 6 kDa small proteins. The *proteasome* recognizes the poly-ubiquitinylated proteins, unfolds and degrades them to small peptides in an ATP-dependent manner.

The Amyloid Structure: the Most Prevalent Structural Disorder

As we described above, the development of the functional 3-dimensional structure is a complicated process which contains many folding traps. Misfolded proteins can be rescued from these traps by the help of molecular chaperones, or can be eliminated by the proteasome. If both fail, damaged proteins form irreversible aggregates, which contribute to the development of lethal diseases. The most prevalent structure of these irreversible aggregates is called "amyloid". Recently it has been increasingly accepted that with a slight modification of physiological parameters, such as the pH or ionic strength, a large number of proteins may form amyloid. The widespread occurrence of amyloids is derived from the general structure of proteins. Two parallel polypeptide chains may attach to each other using a large number of hydrogen-bonds. This *β-structure* can be formed from polypeptides having any amino acid sequence. The *β-structure* is repetitive, which means that the first two polypeptide chains attached to each other in this manner can be a 'seed' of an extremely large protein aggregate, where all chains are in an extended *β-structure* form (*Fig. 1a*). The amyloid aggregate represents a great danger since neither the molecular chaperones nor the proteasome can de-aggregate or destroy it. Therefore, the formation of amyloid fibres in most cases is an *irreversible* process. Given the widespread possibility of the formation of amyloid, as well as the large protein concentration inside the cell that favours aggregation processes, it is rather a miracle that we find amyloid fibres so rarely in nature. This shows the extraordinarily high efficiency of the chaperone and proteasomal systems.

The exact structure of the amyloid resembles that of starch, which is reflected in its name. The amyloid structure has three signature structural features which also allow its identification: (i) Tinctorial properties: the amyloid shows a typical apple-green birefringence, which can be enhanced by Congo red staining; (ii) in an electron microscopic picture amyloids can be detected as 6 to
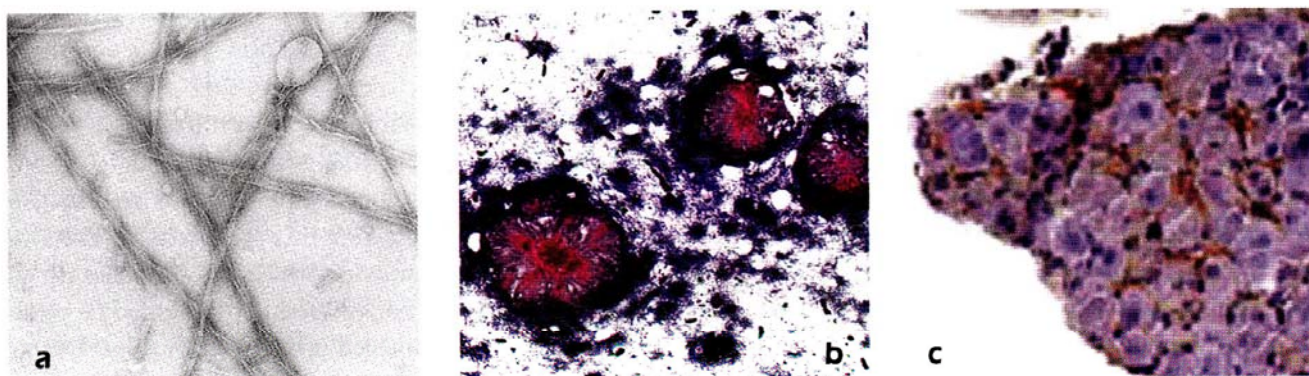
Figure 1. *Electronmicroscopic images of typical amyloid deposits and lesions.*
The ultrastructural image of amyloids is practically independent of the actual precursor protein, being straight fibers of 7-10 nm in diameter (**a**). Their characteristic tinctorial properties and cross-β structure by X-ray diffraction are diagnostic of amyloids (cf. text). Amyloid fibers coalesce into large aggregates, called plaques, which have appearance and tissue localization characteristic of the given disease. The figure shows extracellular plaques characteristic of Alzheimer's disease (**b**), TTR deposits of senile systemic amyloidosis (**c**)

10 nm diameter fibres, whose shape is always the same and is independent of the constituent polypeptide chain (*Fig. 1a*); (iii) the amyloid shows an X-ray scattering typical of cross-β structures.

Amyloid-formation in various amyloidoses has three major origins; all three cause the accumulation of misfolded proteins, leading to their aggregation and formation of the amyloid (*Fig. 2*). Proteins may harbour *mutations*, which may destabilize their native structure, and this helps the accumulation of misfolded folding intermediates. Though amyloidoses are generally lethal diseases, many of them manifest only in elderly people, and thus the underlying protein mutations can be inherited by descendants. These forms of amyloidosis are therefore called (*familial*) amyloidosis. Those mutations, which grossly destabilize the hosting proteins, may induce amyloidosis even in young age. We may thus discriminate young-onset (juvenile) and late-onset (senile) amyloidoses. Grossly destabilizing mutations lead to rapid disease development and to more serious conditions. Several familial amyloidoses are *polygenic* diseases, showing the complexity of the mechanisms promoting and preventing the formation and aggregation of amyloid fibres.

The other usual pathway leading to amyloidosis is the misfolding of a native protein in which all amino acids have been correctly synthesized. If the misfolding happens with an increased probability and/or the chaperone and proteasomal systems became saturated, overloaded for any reason, the initial aggregates may propagate, presenting an even more serious challenge to the chaperones and proteasomes. This vicious cycle may finally lead to the development of *sporadic* amyloidosis. Most sporadic amyloidoses are late-onset diseases which become manifest only in aged people. This is beacuse aged cells contain a large amount of damaged proteins, which increases the 'baseline load' of the chaperone and proteasomal systems. Moreover, in aged cells both chaperones and proteasomes may become damaged themselves, which further incapacitates the defence mechanisms against amyloidosis.

The third underlying reason can be derived from the first two. Some protein aggregates may serve as seeds of further protein aggregation in organisms different from their original host. We should consider these proteins as infectious proteins, and call them *prions*. The initial formation of prions – thus the original amyloidosis – may happen as a spontaneous conformational change, or may proceed as a mutation-induced conformational switch. The propagation of prion-depen-dent aggregation usually uses 'normal', non-mutant proteins which change their conformation and adapt to the amyloid structure with the help of the original prion-seed. It is rather interesting that prions have a normal function in their initial, native conformation. Many prions (especially those in yeast cells) have a physiological function even in their amyloid-prone conformation; thus they serve as molecular switches forming a DNA-independent way of inheritance.

Aggregation may play a dual role in the pathomechanism of amyloidoses. In the first type
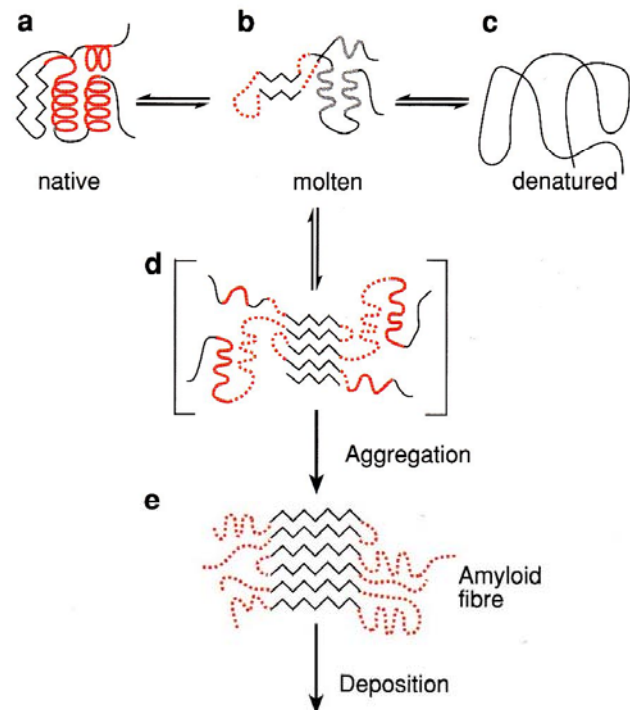
Figure 2. *Scheme of structural changes during amyloid formation.*
The molecular mechanism of amyloid formation can be generalized in the following steps. The protein in its native state has a stable, well-defined structure (**a**). As a result of environmental factors and/or mutations, this structure partially unfolds and gets into a "molten" state (**b**). Formation of amyloid is most likely from this intermediate, molten conformational state, and not from the fully denatured, unfolded state (**c**). In the molten state segments of the polypeptide become connected by H-bonds to neighboring protein molecules (**d**). The formation of this transient aggregated state takes a long time, and is reversible under a critical size. Above the critical size the transition becomes irreversible, and the aggregate keeps extending by capturing additional protein molecules, in the formation of orderly amyloid fibers of repetitive structure (**e**). Fibers are deposited at various points of the body, causing diseases.

the damaged protein cannot maintain its original function, thus the formation of the amyloid results in a *loss-of-function* phenotype. In the case of a heritable amyloidosis, the healthy allele is able to provide the normal function; thus the inheritance shows a *recessive* pattern. However, in most cases, the formation of the amyloid results in the appearance of a novel, damaging function leading to a *gain-of-function* phenotype. This is inherited in a *dominant* manner. The novel function of the amyloid may be manifested as the segregation and inhibition of low-abundance proteins, such as *transcription factors, receptors, signaling proteins*, and thus the rearrangement of the signaling pathways of the host cell.

Kinetics of Amyloid Formation

The formation of the amyloid fibre starts with a conformational rearrangement of the amyloid-forming protein, where the original conformation (whatever it was) is switched to a cross-β structure (*Fig. 2*). This rearrangement is usually blocked or greatly hindered by the stability of the native structure and by the efficiency of the chaperone system. Therefore, the formation of the initial small fibre (*seed*) is the rate-limiting step of the process. If the seed is below a critical size, it is unstable and will dissociate to the monomer proteins. If the aggregation proceeds further, and the seed grows above the critical size, the aggregation process becomes irreversible. The kinetics of the formation of the amyloid can be best described by the kinetic model of the growth of a one-dimensional crystal. For the development of the amyloid a *critical concentration* of the monomers is necessary, and the *in vitro* kinetics shows a typical *lag-phase*, which depends on: (i) the concentration of the monomers, (ii) their amyloid forming potential (e.g. the presence of various mutations); and (iii) external circumstances (e.g. pH, ionic strength, etc.). This kinetic analogy is verified by an experiment where the solution of monomers is seeded with an amyloid fibre, causing a significant shortening or abrogation of the lag-phase. The incubation time (latency) can also be observed *in vivo*, which is thought to be a major cause of the appearance of amyloid fibres only in aged patients. The latency of amyloid formation in the presence of the mutant, amyloid-prone protein is rather typical, and can be explained by this mechanism. The seeding mechanism can be observed *in vivo* most typically with the prion diseases, where the infectious prion protein, with its already altered conformation, serves as a (micro-) seed and accelerates amyloid formation in the infected organism.

Amyloidoses

Having already described the molecular basis of amyloidoses, the aetiology of this disease-group is rather uniform. However, the actual patho-mechanism of each individual disease shows large differences, making classification of amyloidoses necessary. After the primary sites of amyloid deposits, amyloidoses can be classified into two major groups. In *systemic* amyloidoses, the amyloid fibres are deposited in the whole organism. In neural amyloidoses, the primary sites of amyloid formation are the neurons or other cells of the nervous system. Neural amyloidoses can be classified further to three subgroups: (i) traditional amyloidoses (Alzheimer's disease, Parkinson's disease, etc.); (ii) polyglutamine diseases (Huntington disease, Kennedy disease, spinal and bulbal muscular atrophy, spinocerebellar ataxia, etc.); and (iii) prion diseases (Creutzfeldt-Jakob disease, kuru, Gerstmann-Sträussler-Sheinker syndrome, fatal familial insomnia, scrapie disease, etc.). The chapter will detail only the most important examples of these diseases (*Tables 1 and 2*).

Systemic Amyloidoses

History and Classification
Pathological amyloid deposits were first described by the German pathologist Rudolf Virchow, who reported iodine-stainable, starch-like pathological deposits in 1854. Later microscopic experiments using polarized light showed the birefringence of amyloid fibres, which could be greatly enhanced by Congo red staining (1920). This observation later became a diagnostic feature of amyloids. From there on, the Congo red stainable amyloid deposits, which often occupy extremely large areas, have been intensively studied and characterized. In systemic amyloidoses, amyloid fibres appear in practically all tissues, with a paramount expression in the parenchyma causing the inappropriate function of the affected organs.

We know approximately two dozen amyloid-forming proteins (Table 1), which all lead to the deposit of amyloid fibres by the mechanisms out-

Table 1. *Systemic amyloidoses.* Standard nomenclature of amyloidoses, based on the name of the amyloid-forming or precursor protein/peptide, which leads to the formation of systemic amyloid deposits

Amyloid protein/peptide	Precursor	Amyloidosis/primary disease
AL	Immunoglobulin light chain	myeloma multiplex, primary AL amyloidosis
AH	Immunoglobulin heavy chain	myeloma multiplex, primary AH amyloidosis
ATTR	Transthyretin	senile systemic amyloidosis
AA	Serum amyloid A	reactive AA amyloidosis, familial Mediterranean fever
$A\beta_2M$	β_2-microglobuline	hemodyalysis-associated amyloidosis
AApoAI	Apolipoprotein AI	familial systemic amyloidosis
AApoAII	Apolipoprotein AII	familial kidney-amyloidosis
AGel	Gelsolin	familial (Finnish-type) amyloid polyneuropathy
ALys	Lysozyme	familial systemic amyloidosis
AFib	Fibrinogen α-chain	familial systemic amyloidosis
ACys	Cystatin C	familial (Islandic-type) amyloidosis
ACal*	(Pro)calcitonin	medullar thyroid tumor
AIAPP*	Islet amyloid polypeptide	diabetes type II
APro*	Prolactin	hypophysis adenoma
AIns*	Insulin	insulinoma (iatrogen)
ALac*	Lactoferrin	familial corneal amyloidosis
ABri	ABriPP	familial (British-type) dementia

* Sometimes amyloid is preferentially deposited in one organ.

lined in the previous sections. Despite this basic similarity in the underlying patho-mechanism, these proteins do not resemble each other regarding their amino acid sequence or protein folds. Systemic amyloidoses have four groups. We talk about a primer amyloidosis, if the incidence of the disease was apparently without any particular initiating event. We talk about secondary amyloidosis if the protein aggregation was a result of another, primary disease; lowercase such a disease may be tuberculosis, bone infection or arthritis. The third group contains the heritable systemic amyloidoses, which are caused by the mutation of a target-protein. The fourth group contains late-onset diseases, which are caused by long-time high levels of an amyloid-prone protein, such as transthyretin, TTR.

Pathogenetics, Biochemistry and Pathology

The newest classification uses the molecular mechanism as a basis for classification, leading to different groups of these diseases. The starting point of this approach is that all amyloid-forming proteins (Table 1) are present in the body at a permanently elevated level. This has essentially two different origins. In non-heritable amyloidoses, the level of one normal, non-pathological protein increases continuously. We will describe four of these diseases in detail. In *reactive systemic (AA) amyloidosis* caused by acute infections or inflammatory diseases (such as rheumatoid arthritis), an increased level of serum amyloid A (AA) is induced. In these conditions a 76 kDa proteolytic fragment is produced. In approximately 10% of the cases this fragment is aggregated to amyloid fibres, which are primarily deposited in the spleen, kidney and liver. Amyloidoses caused by the monoclonal immunoglobulins (*systemic AL and AH amyloidoses*) usually accompany myeloma multiplex and monoclonal gammopathies. In these diseases the overexpression of a monoclonal antibody is caused by a benign (or in 20–30% of cases

Table 2. *Best known neurodegenerative amyloidoses.* A list of neuron-specific proteins/peptides forming amyloids in the nervous system

Amyloid protein/peptide	Precursors	Mutation	Aggregate	Disease
Ab	APP	APP, PS1, PS2	Senile plaque	Alzheimer's
APrP^{Sc}	Prion protein	Prion protein	Prion aggregate	CJD, other prion diseases
Huntingtin	Huntingtin	PolyQ expansion	Huntingtin inclusion	Huntington's
Androgen receptor	Androgen receptor	PolyQ expansion	AR inclusion	Kennedy's disease, spinal and bulbar muscular atrophy
Ataxin 1	Ataxin 1	PolyQ expansion	Ataxin inclusion	spinocerebellar ataxia
α-synuclein	α-synuclein	α-synuclein, parkin, UCH-L1	Lewy-body	Parkinson's
Tau protein	Tau protein	Tau protein	PHF, Pick-body	Alzheimer's, Taupathies, Pick disease, FTDP-17

malignant) proliferation of one plasma cell clone. The amyloid fibre may be formed from the light (AL) or heavy (AH) chains of the immunoglobulin, and may be deposited in the parenchyma of any tissue except the brain. Additional systemic amyloidoses may be caused by the inefficient degradation and sequestration of certain proteins. The most important example of this deficiency is the *hemodialysis-associated amyloidosis*, which is a secondary effect of acute renal failure, where the level of the surface HLA-I invariant light chain (b_2-microglobulin) is chronically elevated due to the deficient catabolism of this protein by the kidney. The protein cannot penetrate through the dialysis membrane and its concentration becomes extremely high for a long time, which may lead to even lethal defects after the first 5 to 7 years of dialysis treatment. The major symptoms occur in joints and bones. *Senile systemic (ATTR) amyloidosis* is a disease typical of the elderly with a largely elevated prevalence above age 70. The disease becomes typical to most patients above age 85 to 90. The amyloid is formed here from the normal, wild type *transthyretin* (TTR), which is deposited in the capillaries without major symptoms. This capillary-specific amyloid formation, however, may cause a congestive cardiac disorder in the heart.

The other major group of systemic amyloidoses is caused by those destabilizing mutations of the proteins forming the amyloid fibre. The proteins are the same as in the previous forms; however, their amyloid-forming potential is inherited in an autosomal dominant manner, becomes manifest much earlier, and their progression is much faster than

that of spontaneous amyloidoses. These diseases occur most typically as the result of mutations of *transthyretin* (TTR), and may cause *familial amyloid polyneuropathy*, *peripherial neuropathy*, *cardiomyopathy* and *nephropathy* in different patients. More than 80 TTR mutations have been identified worldwide. The best known mutation of another protein, cistatin C (L68Q), leads to *cerebral amyloid angiopathy*, which becomes manifest by systemic deposits of the protein and by repeated minor strokes. Mutations of the apolipoprotein AI and the fibronectin α-chain cause non-neural systemic amyloidosis, appearing in practically each parenchymal tissues, most typically in the kidney. Mutations of the lysozyme enzyme have been analyzed in detail. This antibacterial glycosidase is a key enzyme of defence. Practically, each of its mutations (e.g. I56T, F57I, W64R and D67H) causes destabilization of the compact and stable enzyme structure. In the resulting familial systemic amyloidoses, lethal amyloid deposits appear in the guts, typically causing kidney failure and gastrointestinal bleedings.

An important diagnostic and therapeutic feature of systemic amyloidoses is that the amyloid fibres contain other components such as glucose-amino-glycans and proteo-glycans besides the precursor protein. These molecules bind tightly but non-covalently to the amyloid fibre, and enhance its physical and chemical resistance. The amyloids may also bind a non-fibrillar plasma protein, the serum amyloid P component (SAP), which is important as a therapeutic target and as an *in vivo* diagnostic tool due to its application in ^{123}I-SAP scintigraphy. Some other types of amyloids may also

bind other proteins, such as apolipoprotein E and protease inhibitors.

Therapeutic Possibilities

Locations of tissue deposits of the amyloids and the symptoms of amyloidoses show a great variability. The size of the amyloid fibres can be extremely large, which may hinder the blood supply to adjacent organs or may even cause their physical damage. Moreover, small oligomers or protofibrils of amyloid-forming proteins may disturb normal cellular processes since they may bind to cell surface receptors or ion channels causing the erroneous activation of signaling pathways. Indeed, several observations show that amyloid intermediates are the most toxic forms; and they cause apoptosis, which is in accordance with the lack of inflammatory response in these diseases. (The alternative way of cell death, necrosis, would cause inflammation.) Despite the insensitivity and non-solubility of the amyloid fibres, elimination of their monomer hinders their growth and sometimes induces even their disaggregation. This observation forms the basis of most current successful therapeutic approaches. Before going into the details of therapies applied, we would like to emphasize that the large variability of the underlying causes of amyloidoses, the various tissue-specificities, and the variability of clinical symptoms often make the identification of the primary molecular reason difficult. Without a therapeutic intervention the survival rate of amyloidosis patients may be very low (e.g. in case of AL amyloidosis, the survival may be less than one year).

Having an early diagnosis of the underlying cause at the molecular level is a key factor for a successful intervention. Knowing the molecular source, we may prevent the supply of the amlyoid monomer. Alternatively, we may also resort to the transplantation of the most affected organs. Organ transplantation is a usual solution in case of kidney failure; where a preceding dialysis may also offer a great help. However, liver or heart transplantation may also significantly extend the life of the affected patient. We may have an efficient intervention to slow or even reverse the course of the disease in the case of inflammatory AA amyloidoses. If the primary cause is a chronic infection or tumor (like Castleman disease), surgery or application of appropriate medicines may achieve a complete success. In the case of chronic inflammation (e.g. rheumatoid arthritis, Crohn-disease), the application of non-steroid anti-inflammatory drugs or cytotoxic agents may signifi-

cantly decrease the level of the serum AA amyloid precursor. In summary, in approximately half of these cases clinical intervention may partially or fully reverse the formation of amyloid plaques.

In the systemic amyloidoses caused by myeloma multiplex we need to eliminate the B-cell clone which overproduces the monoclonal immunoglobulin. This may be achieved by cytotoxic agents, corticosteroids, or by autologous stem-cell implantation. Though all these interventions have a rather high risk of toxicity and side-effects, they may significantly increase the survival rate (a typical outcome is an increase of survival from 1 to 5 years). In dialysis-associated amyloidosis decrease of β_2-microglobulin level can only be achieved by kidney transplantation. After performing a successful intervention, the concentration of the precursor immediately decreases, which is followed by a slow regression of the amyloid deposits. A novel therapeutic approach to treat familial transthyretin (TTR) amyloidoses takes into account that the primary source of plasma TTR is the liver. The elimination of the circulating mutant TTR form (e.g. the V30M mutant) can be achieved by liver transplantation, which reverses the formation of amyloid deposits and eases the symptoms, as long as the disease has not reached an advanced stage by the time of transplantation. Since in TTR amyloidoses the liver is not affected by the disease, the liver of the TTR patient can be transplanted to another patient, which is called as 'domino transplantation'. The progression of transthyretin amyloidosis is very slow; therefore, the mutant TTR-producing liver will not cause a disease in the acceptor organism for 15 to 20 years.

Other promising but so far experimental forms of clinical treatments affect the formation of the amyloid fibres directly. Currently, there are a large variety of approaches to inhibit the formation of amyloid fibres, such as the application of glucose-amino-glycan analogues, or β-strand breaker peptides, which prevent the formation of the cross-β structure. Other small molecules (called synthetic chemical chaperones) are also used, which stabilize the non-β conformation of the precursor protein, or help the reversal of amyloid conformation to the native conformation of the protein. In addition, there are ongoing experiments with a large number of chaperone-inducer or chaperone co-inducer molecules. A further, alternative, approach is the prevention of the association of serum amyloid protein (SAP) to the amyloid fibres. Since SAP stabilizes the amyloid fibres by preventing their degradation,

this approach may also yield an efficient method to slow the progress of the disease.

Tissue-Specific Amyloidoses

We usually denote neurodegenerative diseases of the central nervous system as tissue-specific amyloidoses (*Table 2*). Though the major causes of these diseases lie in three different elements, their joint description is justified not only by the common patho-mechanism (the amyloid-dependent cell death), but also by the similar sets of symptoms (movement coordination disfunctions, emotional/psychic disorders, *progressive dementia* and, finally, full mental and physical decay). Another common feature of these diseases is that currently we cannot cure them, but may only offer palliative treatment in some cases.

Alzheimer's Disease

Background
Alzheimer's disease (AD) is a typical senile cortical neurodegenerative disease, one of the best known amyloidoses and one of the first folding disease to be recognized. In 1906, an Austrian physician, Alois Alzheimer, reported that the cortical region of his patients who had died of an unusual psychic illness were covered by white deposits, or plaques. Plaques were surrounded by extensions (dystrophic neurites), within which another typical lesion, paired helical filaments (PHFs), could be observed. As a result of intensive research, many molecular details of the disease are now known. The major component of plaques is compare Table 2pG2 "Ab", generated from a protein termed amyloid precursor protein (APP), and the major component of PHFs is tau, a microtubule-associated protein. Lesions most often appear in the parietal and temporal lobe, accompanied by synaptic degeneration and death of neurons. Granulovacuolar lesions (vacuoles of 5 mm diameter, containing granules) can also be observed. There are some characteristic differences between the diseases that manifests before, or after about 65 years of age.

Pathobiochemistry, Pathogenetics
In about 1–5% of the cases the disease is inherited in an autosomal, dominant fashion, when the mutations typically affect the APP gene (on chromosome 21) and/or presenilin 1 and 2

(PSEN1, chromosome 14, PSEN2, chromosome 1). Under normal physiological conditions APP metabolism is initiated by two proteolytic cleavage events of the protein, which we catalyzed by α-secretase and χ-secretase. It has been shown recently that α-secretase activity is associated with TACE (tumor necrosis factor α-converting enzyme) and ADAM10 (disintergin and metalloproteinase domain 10), whereas χ-secretase activity is associated with multiprotein complexes containing presenilin(s). The product of cleavage at the α- and χ-sites shows no propensity for amyloid formation, i.e., normal processing is inhibitory to amyloid formation. On certain occasions cleavage does not occur at the α- and χ-sites, but at α- and β-sites, due to the action of β-secretase. This cleavage results in the appearance of β-amyloid peptides of 40-42 amino acids in length (Aβ), which are of limited solubility and enhanced amyloidogenicity. The enzyme responsible for β-secretase activity is BACE (β-site amyloid precursor protein-cleaving enzyme), and its action leads to the accumulation of Aβ and its deposition into plaques, which is the causative event in AD. Another element in the aetiology of the disease is the over-activation of certain enzymes, such as Cdk5 (cyclin-dependent kinase 5), which leads to the hyperphosphorylation of tau protein, its dissociation from microtubules and aggregation into PHFs. PHFs, however, are thought to form rather as a consequence of disease, because it also appears in other diseases, *taupathies* and *frontotemporal dementias* (e.g. frontotemporal dementia with Parkinsonism linked to chromosome 17, FTDP-17).

Mutations in the inherited forms (APP, PSEN1, PSEN2) promote formation of Aβ and lead to the disease. In families where one parent has juvenile AD and carries one of these mutations, there is 50% chance that the child will also become affected by AD. Although the frequency of inherited forms overall is low in AD, they had a major role in uncovering the aetiology of the disease. No mutations could be identified in relation to senile forms, but it could be shown that polymorphisms of four different genes [apolipoprotein E (ApoE), α-2 microglobulin, VLDLR (very low density lipoprotein receptor) and LDR (low density lipoprotein receptor-related protein)] represent an increased risk factor. Most information is present on the effects of the polymorphism of ApoE (chromosome 19) because it was shown that the $\epsilon4$ allele occurs in 10–25% of

senile ADs. Its mechanism of contribution is not clear, but it can bind Aβ, thus it is likely to be involved in the formation of plaques. In a few cases AD appears in conjunction with Down syndrome, which is caused by an extra copy of chromosome 21, which leads to a chronic elevation of the level of the encoded APP.

Clinical Pathology, Therapeutic Opportunities

AD is the most prevalent neurodegenerative amyloidosis, with 4 million cases in the USA. It begins with mild memory deficits, problems of spatial and temporal coordination, and a lack of initiative. Solving simple math problems may become difficult. In the middle stages more severe memory deficits develop, with problems of recalling names of family members and everyday words. The patient requires assistance with simple routine tasks of clothing and washing. Psychic problems, such as hallucinations, emotional outbreaks, depression, bewilderment, also appear at this stage. The final stage of the disease brings complete decay of personality; the patient cannot recognize others and requires attention around the clock. Continuous progress of the disease typically leads to death in 8–10 years. Apart from inherited cases, the causes are not known, several primary events, such as inflammation, viral infection, oxidative stress and environmental factors have been implicated. The disease is progressive, with no cure at present. Limited success has been reported in alleviating symptoms, such as the application of antidepressants, mitigating hallucinations to ease psychic problems, improvement of general brain metabolism and blood circulation, and facilitation of neuronal communication, such as by acetylcholinesterase inhibitors, monoamine oxidase (MAO-B) inhibitors, and N-methyl-D-aspartate (NMDA) antagonists. There are several directions of research aimed at finding remedies for AD. One option is the application on non-steroid anti-inflammatory drugs, based on the noted correlation between brain inflammation and the development of AD. Another promising direction is the direct inhibition of the production of Aβ, by virtue of developing β-secretase inhibitors. The deposition of plaques may in principle be directly interfered with, as mentioned in the case of systemic amyloidoses. These molecules may work by stabilizing native structure, or by preventing the incorporation of partially denatured molecules into the aggregate.

Parkinson's Disease

Background

Parkinson's disease is the second most frequent neurodegenerative disease which is caused by protein misfolding, and was first described by James Parkinson in 1817. It affects about 1 million people in the USA. Its causative event is the aggregation of a neuronal protein, α-synuclein, the normal physiologic function of which is not known at present. There are also familial forms of the disease, caused by mutation(s) of the α-synuclein gene. Besides these mutations, the aetiology of the disease may also involve oxidative stress and environmental risk factors, such as the exposure to pesticides and herbicides.

Pathobiochemistry, Pathogenetics

The primary clinical marker of PD is the appearance of specific aggregates, *Lewy-bodies*, in the *dopaminergic* neurons (neurons which use dopamine as neurotransmitter) of *substantia nigra*. Most often the disease appears sporadically, whereas occasionally its senile form is inherited in an autosomal dominant manner. Mutations in these cases affect α-synuclein (on chromosome 4, A53T, A30P, E46K). α-Synuclein can be found primarily in the pre-synaptic terminals of neurons, where it probably regulates the localization of pre-synaptic vesicles, and thus synaptic transmission. Another mutation associated with PD affects UCH-L1 (ubiquitin C-terminal hydrolase L1), which may contribute to the disease by impairing de-ubiquitination of α-synuclein, which might be inhibitory to degradation of structurally compromised α-synuclein by the proteasome. A rare, juvenile form of PD is associated with the autosomal recessive mutation of the parkin gene (chromosome 6). Mutations can be either deletions or point-mutations, in which case Lewy-bodies do not appear in neurons.

Lewy-bodies may also contain chaperones, but their major constituent is α-synuclein, which may also be ubiquitinated and hyperphosphorylated, as well as mutations. The observation that tau fibrils may also be observed in PD (just like in familial AD and Down syndrome) suggests intimate relationships between different conformational diseases. In addition, it may also suggest that α-synuclein and tau may mutually promote and initiate fibril formation by the other.

Clinical Pathology, Therapeutic Opportunities

Toxicity of Lewy-bodies may elicit the death of substantia nigra dopaminergic neurons, which leads to an imbalance of dopamine transmission due to a

reduced stimulation of striatal D1 and D2 receptors. Due to the different functions of receptors, inhibitory effect on thalamus may intensify, whereas excitatory input on cortex and spinal cord may diminish, leading to impairment in movement coordination. The disease usually manifests at about 55–65 years of age, with characteristic symptoms of resting tremor, slowing down of movements (bradykinesia) and slowness in initiating movements (hypokinesia). Short steps, walking with a shuffle and lack of mimicking also appear among the first symptoms. Resting tremor may appear for years on only one side of the body, primarily in the distal parts of limbs. Besides physical problems, PD also has psychic disturbances (anxiety, sleep disorder, depression), and dementia at later stages. From a therapeutic point of view, PD has a special status among neurodegenerative diseases. Because the symptoms all result from a deficiency of dopamine in the striatum, they can be alleviated by treatments that recover normal dopamine level. Some effective drugs promote synthesis of dopamine (dopamine precursors, e.g. L-dopa), others inhibit its degradation (dopamine antagonists, e.g. MAO-B inhibitors). Dopamine effect may also be transiently increased by dopamine agonists, which stimulate D1 and D2 receptors. Cognitive impairment may be counterbalanced by NMDA antagonists, whereas physical symptoms (tremor most of all) can be mitigated by anti-cholinergic drugs. There is no treatment to prevent aggregation of α-synuclein and neuronal death, although there are some hopes of surgical intervention (destroying sub-thalamic regions or implanting fetal substantia nigra cells).

Huntington's Disease

Background

Huntington's disease (HD) is much less prevalent than Alzhemier's or Parkinson's (about 30,000 cases in the USA), but has a special status because it is representative of a unique group of protein aggregation diseases caused by polyglutamine (polyQ) regions of protein (also termed trinucleotide repeats or CAG-repeats). The disease was described in 1872 by G. Huntingtin in Long Island, New York. Polyglutamine diseases, such as Huntington's, Kennedy's, spinal and bulbar muscular atrophy (SBMA), spinocerebellar ataxia (SCA), etc., are progressive, lethal diseases which are always caused by the mutation of a neuronal protein. Their common denominator is the special mechanism of the mutation.

Pathobiochemistry, Pathogenetics

The cause of HD is the mutation of huntingtin, a neuronal protein with transcription regulatory functions. The gene is found on chromosome 4, and its exon 3 encodes for an unusual repetitive polyQ region. In healthy individuals glutamine is repeated 15–36 times, which causes no problem. The highly repetitive nature of this region, however, poses particular danger because the CAG repeats encoding for glutamines are liable to form hairpin structures during DNA replication, which causes replication slippage and an extension of the repeat region. In addition, longer regions are more prone to extension, i.e., the extension tends to be self-propagating. In some individuals the repeat number may be as large as 120, the deleterious effect of which comes from the fact that repeats above 40 glutamines have a reduced solubility and tendency to aggregate. Aggregation is correlated with the physical properties of the polyQ region, inasmuch as it has a random coil structure below 40, but gets stabilized in an insoluble β-structure when it becomes longer. It often happens in HD that the number of glutamines increases with every generation, in parallel with the manifestation of the disease at an ever younger age, a phenomenon which is termed anticipation. Due to anticipation, the incidence of juvenile HD is about 10%, and it can manifest anytime before the age of 20.

The neuropathological basis of the disease is the deposition of aggregates within neurons, which causes cell death. It may be the consequence of the translocation of aggregates to the nucleus, and binding of specific transcription factors, such as CREB-binding protein (CBP). Cell death in both adult and juvenile forms is most severe in basal ganglia and putamen areas. In juvenile forms Purkinje cells of the cerebellum, nucleus dentatus and hippocampus may also be affected. Striatal degeneration appears to be correlated with the severity of symptoms.

Clinical Pathology, Therapeutic Opportunities

Huntington's disease usually manifests around the age of 40–45, with clinical symptoms that belong to three groups. Problems of coordination of movement may be rigidity (rigor), unintentional, involuntary movements (hyperkinesis), which may develop into the inability to speak and swallow at later stages of the disease. Uncontrolled movements are also termed Saint Vitus dance (chorea). The symptoms are also associated with cognitive problems (dementia), problems with planning and decision-making, as well as spatial coordination. Sharing

attention between different things may also cause problems. As the disease progresses, a decay of communication skills and insistence on everyday routine can be observed. Personality changes may also be severe, such as suspicion, paranoia, outbreaks of temper, anxiety and mania. HD usually leads to death in about 15 years, with the immediate cause pneumonia and/or heart failure. Juvenile HD may have significantly different symptoms: instead of chorea there may be clumsiness, rigor and tremor, and infrequently, repetitive seizures. Because extension of the polyQ regions leads to younger manifestations, in juvenile forms the typical number is on the order of 80–100, which is also associated with fast progress and death within 10 years.

HD has no cure, and only limited treatment of symptoms, such as chorea, psychosis and depression, is possible. One of the most severe symptoms of HD, depression, can be effectively treated with anti-depressants. Apathy can be treated with psychostimulants (e.g. Ritalin). Anxiety can be alleviated by elevating the level of serotonin that can be achieved by selective serotonin re-uptake inhibitors (SSRI), for example. Research is ongoing in several directions to find treatments to slow down progress of the disease. Somewhat paradoxically, inhibition of cellular chaperone Hsp90 by geldanamycin may activate heat-shock factors involved in the synthesis of chaperones, which may be inhibitory to intracellular aggregation of huntingtin. Another opportunity is the application of anti-inflammatory drugs since chronic neuronal inflammation appears to be important in mediating neuronal death in HD. Correspondingly, some glucocorticoids are in clinical trials in both AD and HD. Free radicals may also contribute to HD aetiology. Antioxidants, such as coenzyme Q10 and vitamin E, are also under trial. Antagonists to glutamate excitotoxicity and effectors of neuronal transcription also bear the promise of therapeutic effect.

Prion Diseases

Background

The prion disease of sheep (scrapie) has been known for some 200 years. Another similar disease, kuru, was endemic among people of the Fore tribe of Papua New Guinea, and reached epidemic proportions in the first half of the 20th century. The cause of this epidemic was ritual cannibalism, in which people consumed the brains of their deceased relatives. The major symptoms of kuru are grinning grimaces on the face, gasping for breath and patho-

logic bursts of laughter in the terminal stages of the disease. Thus it is also termed "laughing sickness". Although the mechanism on transmission was not initially clear, the fact that its incidence started to decline after the ban of cannibalism by missionaries proved that infection was the cause. The disease was critically studied by Charleton Gajdusek, who suggested that, given several years of incubation, the disease is caused by a pathogen of a novel type, termed slow virus. Another known prion disease is Creutzfeldt-Jakob disease (CJD), described in 1920 by Hans Gerhard Creutzfeldt and in 1921 by Alfons Maria Jakob. Further human prion diseases (fatal familial insomnia, FFI; Gerstmann-Sträussler-Scheinker Syndrome, GSS) are less frequent and less well known.

Attention has been directed to ward prion diseases by an outbreak of mad-cow disease, which is a form of transmissible spongiform encephalopathy, termed bovine spongiform encephalopathy (BSE). A large part of the cow population in Great Britain had to exterminated because it has been suspected that a novel juvenile variant of CJD, vCJD, is caused by the human consumption of meat of infected but symptom-free animals. This suspicion was later verified. Although the number of fatalities from vCJD is rather low, the analysis of surgical biopsies suggests that several thousand people may already be infected.

The mechanism of the unusual behavior of prion was elucidated by the Nobel laureates Gajdusek and Stanley B. Prusiner. It was Gajdusek who recognized the similarities in the pathology of seemingly unrelated disorders scrapie, kuru and CJD, and initiated the search for the pathogen thought to be a slow virus at that time. Identification of the pathogen became possible by the suggestion of the "prion" hypothesis by Prusiner. Based on a range of observations on the unusual resistance of the pathogen to UV irradiation, DNAse treatment, heat treatment and chemical sterilization, and isolation of an infectious agent devoid of DNA and RNA, he suggested that the agent responsible for the disease is a protein. He laid down the "protein only" hypothesis, which forms the basis of the idea that the disease is caused by a proteinaceous infectious particle, or prion.

Pathobiochemistry, Pathogenetics

There is ample experimental evidence that prion is an infectious amyloid which instructs the normal cellular form of the same protein in the host to convert to this pathological conformation. The

normal form of prion protein is termed cellular (PrP^C), whereas the pathological form is scrapie (PrP^Sc). Analysis of the two forms suggests that the infectious agent is also encoded in the host organism (on chromosome 20), and there is no difference between them at the level of sequence or post-translational modification. Difference in proteolytic sensitivities and secondary structural propensity (α-helix in PrP^C, β-strand in PrP^Sc) suggests that the cause of the disease is the conformational change of PrP, which may be caused by a mutation, or may be sporadic. Unique to this disease is infectivity, i.e., transmissibility of the prion from one organism to the other. Several dozen mutations of PrP have been identified in inherited forms (*Fig. 3*). Infected material gets into an organism in food, and through the Mucose-associated-lymphoid tissue (MALT) it gets into the lymphocytes, and then through lymphatic system (lymph node, spleen and tonsil) it eventually arrives at the nervous system. A conclusive evidence for this series of events is that SCID mice, and homozygous prion null-mutant (PrP^{0/0}) mice cannot be infected by prions. Infection may be caused by alimentation (animal feed, or consumption of food of animal origin), and by surgical intervention (iatrogenic disease, caused by implantation of infected electrode, growth-hormone preparation, or cornea transplantation).

There are two current hypotheses with respect to the cause of the conformational change. Both assume physical contact of PrP^C and PrP^Sc, and emphasize the autocatalytic nature of the transition. In the *template-assisted* model, PrP^Sc is assumed to be thermodynamically more stable than PrP^C, but as the transition is kinetically inhibited, it can only occur in the presence of PrP^Sc as a catalyst. In the *nucleation-polymerization* model, PrP^Sc is assumed to be less stable, and can only be stabilized when bound to the amyloid form. The two models differ only in these molecular details, both are consistent with the observed kinetic course of the disease. An intriguing observation with respect to the conformational change accompanying infection is the "species barrier", which appears to hinder transmission of the prion from one species to another. Intriguingly, transmission is possible if the recipient carries the transgene encoding for the prion of the infecting species. The most likely explanation of these observations is the lack of productive physical interaction between the scrapie prion and prion protein of the host. It is of note that chaperones also take part in the conformational transition.

Clinical Pathology, Therapeutic Opportunities

Although human prion diseases are all caused by the conformational change of the very same protein (PrP), there are several different prion diseases (*Table 2 and Fig. 3*), such as CJD, GSS, FFI and kuru. The explanation of this apparent contradiction is that there are several different amyloid (prion strains) and the stability of these forms as maintained in a similar way to viral strains. Although each different strain causes spongiform degeneration in the brain, the actual neuronal lesions show characteristic differences. Resulting symptoms, latency of the disease and the time course of the diseases are different. Basic CJD manifests between the age of 50 and 75, with symptoms resembling those of other conformational diseases, such as physical problems (problems in movement, coordination, impaired reflexes, rigor, palsy), cognitive impairment (progressive dementia), psychic disorders (anxiety, derangement). Progress is usually fast, and leads to death within one year after the first symptoms, usually elicited by pneumonia. GSS usually occurs later than CJD, and with less severe symptoms and dementia; it can last 3–7 years. Juvenile CJD, vCJD, on the other hand, manifests much earlier, even below 20 years of age, and it progresses much faster, and may cause death within 2.5–6 months. The first symptoms are personality disorders, bizarre senses of pain, dementia and depression. Progression brings about muscle spasms and ataxy. Major pathological lesions are spongiform degeneration, reduction in neuron number and deposition of prion aggregates. Either way, prion diseases at present are incurable, and they can only be prevented in the case of the iatrogenic version, and by prenatal screening in the case of inherited forms.

Physiological Prions

Amyloids are proteins of permanently changed structure, and may cause disorder by either of two mechanisms: the loss on normal function, or the gain of pathological function. The gain of novel function by the formation of amyloid may, in principle, be also advantageous, which raises the opportunity of normal, functional amyloids. Although it might sound bizarre given the incurable nature of prion diseases, there is evidence for the existence of physiological prions in yeast and in higher organisms, and it may turn out to be a rather general phenomenon in the living world. Physiological

prions *(Table 3)* may impart selection advantages under certain conditions, which has resulted in their observed evolutionary conservation. The first physiological prion to be recognized corresponds to the genetic element [URE3], which is inherited in non-Mendelian fashion. It can be transmitted from one cell to another by cytoduction (injection of the cytoplasm of one cell into the other), and can be "cured", i.e., the phenotypic changes can be reversed by mild detergent treatment. These and other observations have led to the recognition that the heritable element is an amyloid formed by the altered conformational state of protein Ure2p. The phenotypic consequence of transition to the amyloid state is that the yeast cell becomes able to thrive on a given nitrogen source (like urea) in an otherwise nitrogen-free environment.

The other well-characterized case of physiological yeast prion is the genetic element [PSI+], which is the amyloid form of the translation terminator protein Sup35. Because the protein is a component of the translation termination complex, its transition to the amyloid state leads to translation read-through at stop codons. Intriguingly, formation of Sup35 amyloid is facilitated by two other prions, [PIN+] and [NU+], which correspond to proteins Rnq1p and New1p respectively. The action of these "co-prions" resembles the aforementioned mutual facilitation of amyloid formation by mammalian α–synuclein and tau protein. A probably even more intriguing observation relates to the information-carrier properties of amyloid, as suggested in the case of CPEB (cytoplasmic polyadenylation-element binding protein) in the marine snail *Aplysia californica*. The amyloid form of this protein promotes polyadenylation of dormant cytoplasmic mRNA, which contributes to specific changes in local synaptic translation and permanent modification of synaptic communication, which are the basic mechanisms underlying memory.
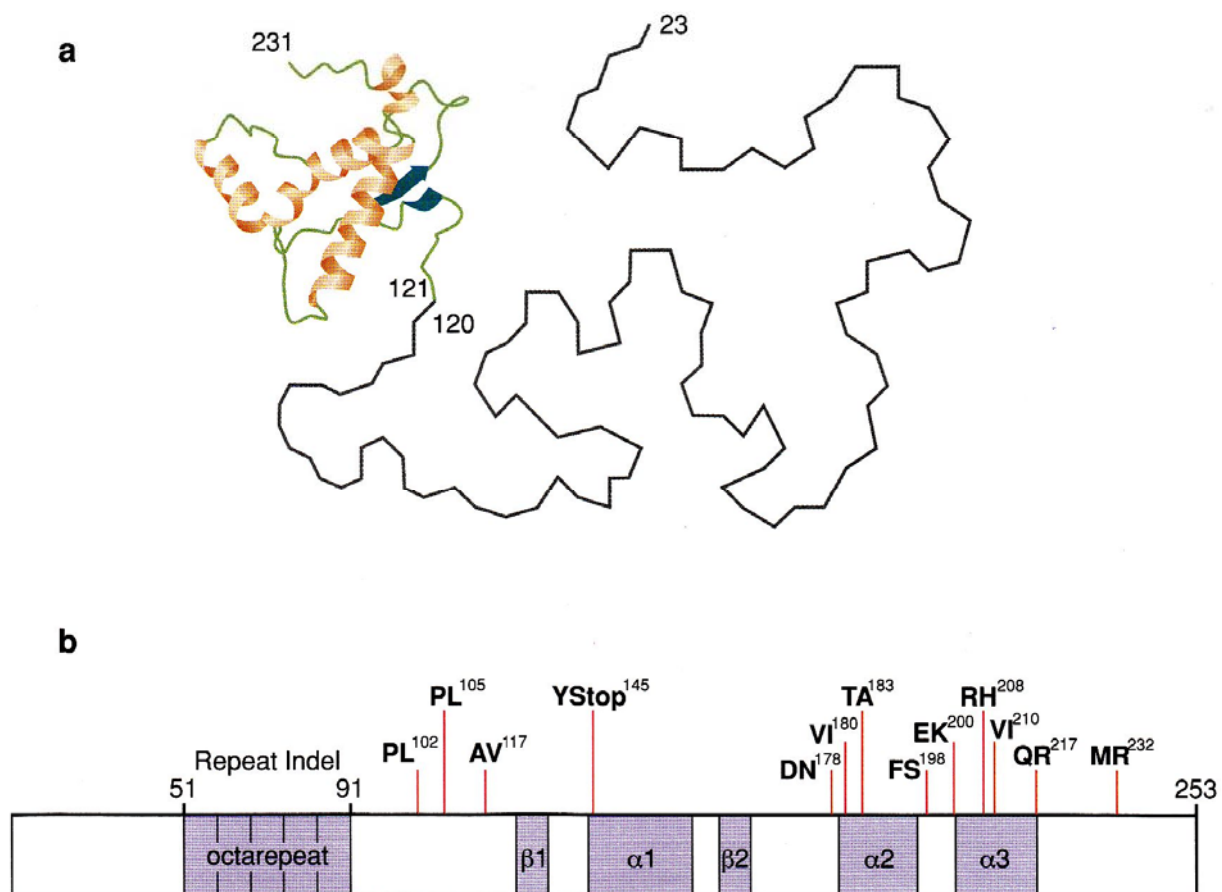
Figure 3. *Structure of prion protein and major mutations in familial prion diseases.* (a) Mature prion protein consists of an N-terminal disordered region (23-121) and a C-terminal half (122-231) of well-defined, mostly α-helical structure. (b) Mutations associated with the inherited forms of the disease cluster in two helices of the ordered region (α2 and α3), but mutations in the disordered N-terminal half (indel mutations of the octarepeat region and point mutations of the conserved region) have also been observed.

Table 3. *Physiological amyloids (prions). The table lists prions, the amyloid form of which causes no disease, but imparts selectively advantageous phenotypic changes*

Genetic element	Prion protein	Species	Function
[URE3]	Ure2p	S. cerevisiae	urea transport/metabolism
[PSI⁺]	Sup35p	S. cerevisiae	regulation of translation
[PIN⁺]	Rnq1p	S. cerevisiae	regulation of prion formation
[NU⁺]	New1p	S. cerevisiae	regulation of prion formation
[Het-s]	HET-s	P. anserina	heterokaryon incompatibility
CPEB	CPEB	A. californica	regulation of translation, memory

Besides their theoretical interest, the observation of physiological prions is also of immense practical importance. Because these prions are found in organisms which are easy to manipulate, their studies substantially promote understanding of the pathomechanism of human amyloidoses. In this way, their study may be instrumental in devising strategies for prevention of amyloid formation, and finding remedies in those case when the disease has already begun.

MEDICAL PATHOBIOCHEMISTRY

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