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Molecular Pathogenesis of Protein Misfolding Diseases: Pathological Molecular Environments Versus Quality Control Systems Against Misfolded Proteins

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Diverse human diseases, including various neurodegenerative disorders and amyloidoses, are thought to result from the misfolding and aggregation of disease-causative proteins, and thus are collectively called protein misfolding diseases. Natively folded disease-causative proteins generally undergo a β-sheet conformational transition through an energetically unfavourable process, and further polymerize into amyloid fibrils. In the case of β_2-microglobulin-related amyloidosis, an extracellular protein misfolding disease, many kinds of biological molecules including glycosaminoglycans, proteoglycans and lipids partially unfold β_2-microglobulin and catalyse its subsequent nucleus formation. After amyloid fibrils are formed, these biological molecules stabilize the β_2-microglobulin fibrils. In the polyglutamine neurodegenerative diseases, an intracellular protein misfolding disease, molecular chaperones as well as the ubiquitin–proteasome and autophagy–lysosome protein degradation systems, which are called the protein quality control systems, strictly regulate protein misfolding, aggregation and disease progression. A family of extracellular chaperones also binds to misfolded proteins and inhibit amyloid fibril formation in the extracellular space. Protein misfolding and aggregation may be an ideal therapeutic target for protein misfolding diseases in general.

Key words: β_2-microglobulin-related amyloidosis, chaperones, polyglutamine diseases, protein degradation system, protein misfolding diseases.

Abbreviations: AA, amyloid protein A; AApoAII, apolipoprotein A-II-related; Aβ2M, β_2-microglobulin-related; α_2M, α_2-macroglobulin; apoE, apolipoprotein E; β2-m, β_2-microglobulin; GAG, glycosaminoglycan; HD, Huntington's disease; Hp, haptoglobin; LPA, lysophosphatidic acid; LPL, lysophospholipid; NEFA, non-esterified fatty acid; PG, proteoglycan; polyQ, polyglutamine; SBMA, spinobulbar muscular atrophy; SCA, spinocerebellar ataxia; UPS, ubiquitin–proteasome system.

Diverse human diseases, including various neurodegenerative disorders and systemic amyloidoses, are thought to result from the misfolding and aggregation of disease-causative proteins either intracellularly, extracellularly or both, and thus are collectively called protein misfolding diseases (*1*). Recent experimental evidence suggests that protein misfolding and aggregation are influenced by various factors including mutations in proteins (*2*) and interactions of proteins with pathological molecular factors/environments (*3*), as well as by intracellular and extracellular quality control systems against misfolded proteins (*4–6*). In this minireview, we will focus our attention on β_2-microglobulin-related (Aβ2M) amyloidosis (*7*) and the polyglutamine (polyQ) neurodegenerative diseases (*8*) as representatives of extracellular and intracellular protein misfolding diseases, respectively, and briefly discuss the roles of the above-mentioned factors and protein quality control systems in the development of protein misfolding diseases.

GENERAL MECHANISMS OF PROTEIN MISFOLDING, AGGREGATION AND AMYLOID FIBRIL FORMATION

In the pathogenesis of protein misfolding diseases, the structural changes of disease-causative proteins can be divided into two steps, *i.e.* a nucleus formation step and a fibril extension step (Fig. 1). Although the precise mechanism of the former is still controversial (Fig. 1A), such disease-causative proteins with native structures generally undergo a β-sheet conformational transition through an energetically unfavorable process (*9*). During the transition, unstable intermediates may be formed (*9*). Various mutations in the disease-causative proteins (*e.g.* amino-acid substitutions or polyQ expansion), as well as interactions of the proteins with various biological molecules may reduce the free-energy barrier(s), facilitating the misfolding of disease-causative

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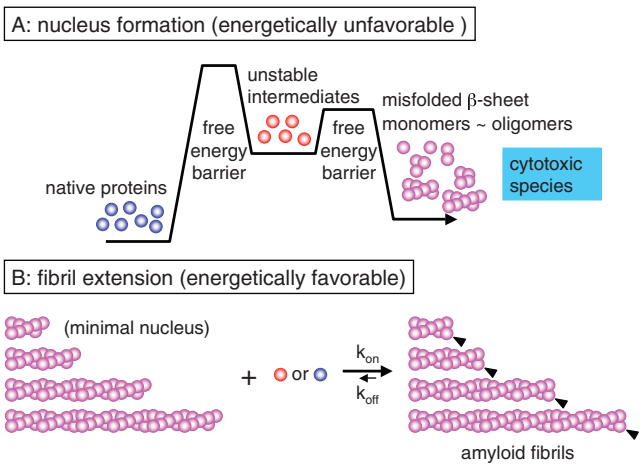

Fig. 1. **A working model for protein misfolding, aggregation and amyloid fibril formation.** (A) Natively folded disease-causative proteins responsible for protein misfolding diseases generally undergo a β-sheet conformational transition through an energetically unfavorable process. During the transition, unstable intermediates may be formed. Various mutations in the disease-causative proteins (*e.g.* amino-acid substitutions or polyQ expansion), as well as interactions of the proteins with various biological molecules may reduce the free-energy barrier(s), facilitating the misfolding of disease-causative proteins. Recent experimental evidence has indicated that misfolded β-sheet monomers or oligomers are the toxic moieties primarily responsible for the development of protein misfolding diseases. (B) Once the minimal nucleus has been formed, further addition of monomers to the nucleus becomes energetically favourable, resulting in rapid extension of amyloid fibrils according to a first-order kinetic model, *i.e.* via the consecutive association of precursor proteins onto the ends of existing fibrils. Arrowheads indicate the misfolded proteins freshly polymerized onto the fibril ends.

proteins (*2*, *10–12*). For example, we have recently demonstrated that mutational expansion of the polyQ stretch triggers a conformational transition to a β-sheet-rich structure, resulting in its assembly into β-sheet-rich amyloid-like fibrils (*11*). Matsuzaki and colleagues (*12*) reported that association of amyloid-β peptide with GM1 ganglioside induces its β-sheet conformational transition, which facilitates amyloid fibril formation.

Recent experimental evidence has indicated that misfolded β-sheet oligomers or even monomers are the toxic moieties primarily responsible for the development of protein misfolding diseases (*13*). Actually, by microinjecting various polyQ protein conformers into cultured cells, we have revealed that the soluble β-sheet monomer causes cytotoxicity (*11*).

In many cases, misfolded proteins further polymerize into amyloid fibrils, and this mechanism has been well elucidated (Fig. 1B). Once the minimal nucleus has been formed, further addition of monomers to the nucleus becomes energetically favourable, resulting in rapid extension of amyloid fibrils according to a first-order kinetic model, *i.e.* via the consecutive association of precursor proteins onto the ends of existing fibrils (*14*, *15*). We have proposed that this nucleation-dependent polymerization model can explain the general mechanisms of amyloid fibril formation *in vitro* as well as in various types of human and murine amyloidoses (*15*). This model has recently been evidenced *in vivo* by the fact that apolipoprotein A-II-related murine senile (AApoAII) and amyloid protein A (AA) amyloidoses are markedly accelerated when the animals are given an intravenous injection or oral administration of AApoAII and AA amyloid fibrils, respectively (*16*, *17*).

PATHOLOGICAL MOLECULAR ENVIRONMENTS INDUCING β_2-MICROGLOBULIN-RELATED AMYLOIDOSIS

Aβ2M amyloidosis is a common and serious complication in long-term haemodialysis patients (*7*). Although the retention of intact β_2-microglobulin (β2-m), the precursor protein of this amyloidosis, in the plasma appears to be a prerequisite (*18*), the mechanism of the deposition of these amyloid fibrils is not fully understood.

In the mechanism of amyloidogenesis from natively folded proteins such as β2-m and transthyretin, their partial unfolding is believed to be a prerequisite for their assembly into amyloid fibrils both *in vitro* and *in vivo* (*9*). The extension of Aβ2M amyloid fibrils *in vitro* is optimum at pH 2.5 and does not occur at neutral pH (*3*). Rather, Aβ2M amyloid fibrils readily depolymerize into monomeric β2-m at neutral pH (*3*). At pH 2.5, β2-m loses much of its secondary and tertiary structures observed at pH 7.5 (*3*). Once incorporated into Aβ2M amyloid fibrils at pH 2.5, β2-m becomes highly rich in β-sheet structure and gains secondary and tertiary structures strikingly different from monomeric β2-m at both pH 7.5 and 2.5 (*3*). To elucidate the molecular pathogenesis of Aβ2M amyloidosis, we considered it essential to identify biological molecules that induce and enhance the formation of Aβ2M amyloid fibrils as well as stabilize them at neutral pH.

Aβ2M amyloid deposition takes place predominantly in the cartilaginous and tendinous tissues (*7*), suggesting that the specific interaction between β2-m and extracellular matrix molecules in these tissues, such as type I collagen, glycosaminoglycans (GAGs), and proteoglycans (PGs), causes Aβ2M amyloid deposition. We reported that various types of GAGs and PGs, as well as apolipoprotein E (apoE), a representative amyloid-associated protein stabilize Aβ2M amyloid fibrils and inhibit their depolymerization at neutral pH (*3*) (Fig. 2). We also reported that some GAGs, especially heparin, dose-dependently enhance the 2,2,2-trifluoroethanol-induced fibril extension at neutral pH (*3*). Relini *et al.* (*19*) indicated that type I fibrillar collagen plays a crucial role in β2-m amyloid deposition under physiopathological conditions, and suggested an explanation to the strict specificity of Aβ2M amyloidosis for the tissues of the skeletal system.

Various lipid molecules have been reported to induce conformational changes of amyloid precursor proteins, as well as to initiate their amyloid fibril formation *in vitro* (*20*, *21*). Lysophospholipids (LPLs) and non-esterified fatty acids (NEFAs) are candidate biological molecules that induce Aβ2M amyloid fibril formation at neutral pH. LPL is a biologically active, proinflammatory molecule with a glycerol backbone to which one hydrophobic fatty acid chain and a hydrophilic phosphate or

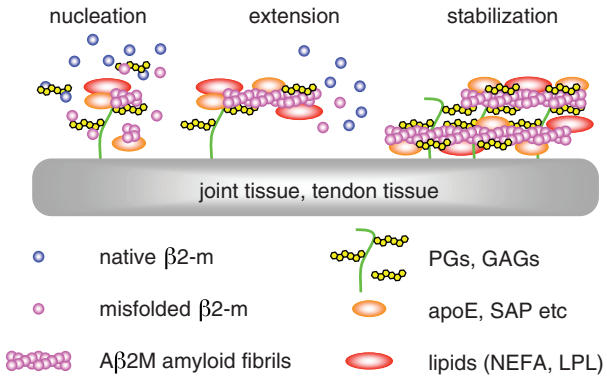

Fig. 2. **A model for the molecular mechanism of Aβ2M amyloid fibril deposition *in vivo*.** Aβ2M amyloid fibril formation can be explained by a nucleation-dependent polymerization model. Partial unfolding of β2-m molecules and subsequent nucleus formation from them are catalysed by many kinds of biological molecules including GAGs, PGs and lipids (NEFA, LPL). After Aβ2M amyloid fibrils are formed, various biological molecules including apoE, GAGs, PGs and lipids form a stable complex with the fibrils, stabilize them and protect them from proteolysis *in vivo*.

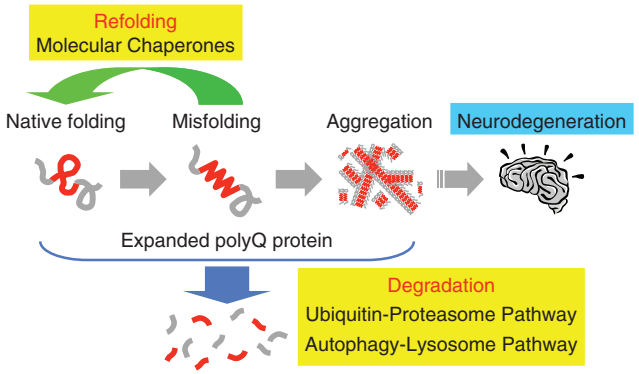

Fig. 3. **Molecular pathogenesis of the polyglutamine diseases and the protein quality control system.** In the pathogenesis of the polyQ diseases, expansion of the polyQ stretch triggers misfolding of the mutant proteins, leading to their aggregation, eventually resulting in neurodegeneration. As defence systems against protein misfolding and aggregation, which are called the protein quality control system, molecular chaperones assist the refolding of misfolded and/or aggregated proteins, and protein degradation systems including the ubiquitin–proteasome and autophagy–lysosome pathways eliminate them.

phosphorylated alcohol is attached. Recently, we reported that some LPLs, especially lysophosphatidic acid (LPA), as well as NEFAs induce the extension of Aβ2M amyloid fibrils at neutral pH, by partially unfolding the compact structure of β2-m to an amyloidogenic conformer, as well as by stabilizing the extended fibrils (*22*, *23*) (Fig. 2). We also observed that haemodialysis patients have significantly higher plasma concentrations of LPA than healthy subjects and that patient plasma samples with the highest LPA concentrations stabilize Aβ2M amyloid fibrils more potently than normal plasma samples (*22*). Moreover, in patients receiving haemodialysis regularly, administration of heparin results in an acute increase in plasma NEFA, largely by the activation of lipoprotein lipase (*24*). These results suggest possible roles of LPLs and NEFAs in the development of Aβ2M amyloidosis. The serum levels of β2-m in patients with end stage renal failure increase from about 0.1 μM to >5 μM (*18*). Thus, the pathological interaction between β2-m and other molecules including LPLs and NEFAs plays a significant role only in haemodialysis patients, resulting in the manifestation of Aβ2M amyloidosis after a long incubation period (Fig. 2).

POLYGLUTAMINE DISEASES AND THE INTRACELLULAR PROTEIN QUALITY CONTROL SYSTEM

PolyQ diseases are a group of hereditary neurodegenerative diseases including Huntington's disease (HD), spinocerebellar ataxias (SCA1, 2, 3, 6, 7, 17), dentatorubral–pallidoluysian atrophy and spinobulbar muscular atrophy (SBMA), which are commonly caused by abnormal expansions of the polyQ stretch (>35–40) within otherwise unrelated disease-causative proteins (*8*). The expanded polyQ stretch renders the mutant proteins prone to be misfolded, leading to a conformational transition of the polyQ stretch to a β-sheet-rich structure and its subsequent aggregation into amyloid-like fibrils, resulting in their accumulation as inclusion bodies in affected neurons and eventual neurodegeneration (*11*) (Fig. 3). It is generally accepted that misfolding and aggregation of the mutant proteins play a pivotal role in the pathogenesis of the polyQ diseases. Indeed, we previously showed that QBP1 (SNWKWWPGIFD), a peptide which specifically binds to the expanded polyQ stretch, inhibits its β-sheet conformational transition *in vitro*, resulting in suppression of polyQ-induced neurodegeneration *in vivo* (*25*, *26*).

To cope with protein misfolding and aggregation, cells have their own protein quality control systems, which consist of molecular chaperones and protein degradation systems (Fig. 3). Molecular chaperones assist not only the folding of newly synthesized proteins but also the refolding of misfolded and/or aggregated proteins (*4*). The first report on the relationship between the polyQ diseases and molecular chaperones came from Zoghbi's group in 1998, in which they discovered that Hsp40 accumulates in polyQ protein inclusions and that expression of Hsp40 suppresses inclusion body formation in a cell culture model of SCA1 (*27*). Subsequent studies also showed that expression of molecular chaperones such as Hsp70 and Hsp40 suppresses polyQ-induced neurodegeneration in *Drosophila* and mouse models (*28*, *29*). In addition, other molecular chaperones such as Hsp27, Hsp84, Hsp105 and more recently, TRiC/CCT were also reported to suppress polyQ-induced cytotoxicity in cell culture. These reports accelerated the search of drugs inducing the expression of endogenous molecular chaperones as promising therapeutic candidates. In 2001, Wanker and colleagues (*30*) demonstrated that geldanamycin, an HSF1-activating compound, suppresses aggregation of polyQ-expanded huntingtin, the responsible protein for HD, through induction of endogenous molecular chaperones in cell culture. Furthermore, Katsuno *et al*. (*31*) reported that geranylgeranylacetone,

a molecular chaperone inducer widely used as an anti-ulcer drug, suppresses neuromuscular phenotypes of a mouse model of SBMA. We also demonstrated recently that 17-(allylamino)-17-demethoxygeldanamycin, a less-toxic derivative of geldanamycin, suppresses neurodegeneration in *Drosophila* models of both HD and SCA3, suggesting its effectiveness against various polyQ diseases (*32*).

Another defence system to deal with misfolded and/or aggregated proteins is the protein degradation system for their destruction (*5*). Two major pathways for protein degradation are the ubiquitin–proteasome and autophagy–lysosome pathways (Fig. 3). The ubiquitin–proteasome system (UPS) degrades short-lived and misfolded proteins, in which substrate proteins are specifically recognized with the help of the ubiquitin conjugation system and targeted to the proteasome for degradation, which requires deubiquitination and unfolding of substrate proteins. In 1997, polyQ protein containing inclusions were discovered for the first time in the brains of HD patients as well as a HD mouse model, which were frequently ubiquitinated like various protein inclusions in other neurodegenerative diseases (*33*). Proteasome subunits were also found to accumulate in polyQ inclusion bodies (*27*). These observations, together with the experimental findings that inhibition of the proteasome results in increased aggregation of the polyQ protein, raise the possibility that impairment of UPS function could be involved in the pathogenesis of the polyQ diseases. In 2001, using the GFPu reporter system, expression of the expanded polyQ protein was found to impair proteasome activity in cell culture models (*34*). However, subsequent studies have failed to confirm UPS impairment in mouse models of the polyQ diseases (*35*). Recently, conditional knock-out of the 26S proteasome subunit in the mouse brain was shown to result in intraneuronal inclusions and neurodegeneration, although its relevance to human neurodegenerative diseases remains controversial (*36*). Moreover, expression of the quality control ubiquitin ligase CHIP was demonstrated to enhance the ubiquitination and degradation of various polyQ proteins and to exert therapeutic effects in *Drosophila* and zebrafish polyQ disease models (*37*). Other ubiquitin ligases such as UFD2a, Hrd1 and E6-AP, were also reported to enhance the ubiquitination and degradation of various polyQ proteins.

The autophagy–lysosome system is a non-selective bulk degradation system for long-lived and misfolded proteins (*38*). Autophagy (macroautophagy) involves engulfment of substrate proteins and/or organelles into the autophagosome and its delivery to the lysosome, which, in contrast to the UPS, does not require unfolding of substrate proteins. In 2000, DiFiglia and colleagues (*39*) first reported that mutant huntingtin expressed in cultured cells accumulates in autophagosomes and activates autophagy. Subsequently, Rubinsztein and colleagues (*40*) demonstrated that expanded polyQ proteins are degraded by autophagy in cell culture, and that rapamycin and its derivative CCI-779, autophagy-inducing compounds, suppress the neurological phenotypes of *Drosophila* and mouse models of HD. Since the autophagy degradation system is considered to be non-selective, it is noteworthy that very recently, specific acetylation of polyQ-expanded huntingtin was found to target it for efficient autophagic degradation (*41*). Blocking autophagy activity in the brain by using conditional knock-out mice was also reported to result in ubiquitinated protein inclusions and neurodegeneration, although it remains unsolved whether the autophagy–lysosome degradation pathway is damaged in the polyQ disease pathogenesis (*42*, *43*).

EXTRACELLULAR PROTEIN QUALITY CONTROL SYSTEM SUPPRESSING AMYLOIDOSIS

It has been proposed that proteins are able to aggregate and form extracellular amyloid deposits *in vivo* when the normally efficient protein quality control machinery is overwhelmed. Clusterin, α_2-macroglobulin (α_2M) and haptoglobin (Hp) are all abundantly secreted glycoproteins present in human plasma and cerebrospinal fluid. Like the small heat shock proteins, all of these glycoproteins have in common the ability to protect a range of proteins from stress-induced aggregation in an ATP-independent manner and have been described as extracellular chaperones (*6*). Interestingly, these glycoproteins have been found associated with amyloid deposits in Alzheimer's disease and many other human amyloidoses. Using an array of biophysical techniques, Wilson's group established that all of these glycoproteins inhibit the formation of amyloid fibrils from a range of proteins *in vitro* at substoichiometric levels and under physiological conditions (*6*, *44*, *45*). They also provided evidence that clusterin, α_2M and Hp interact with pre-fibrillar species to maintain the solubility of amyloidogenic proteins. This is consistent with the hypothesis that these glycoproteins bind to species sharing common structural features present during amyloid formation of a range of substrate proteins. Together with previous findings, Wilson's group suggested that clusterin, α_2M and Hp make up a small family of extracellular chaperones that may be an important part of an *in vivo* quality control system for extracellular proteins. They proposed that extracellular chaperones respond to misfolded and aggregated proteins in the extracellular space by binding to their exposed hydrophobic regions, maintaining the solubility of the substrate, and promoting its removal from the extracellular space via receptor-mediated endocytosis (*e.g.* via low density lipoprotein receptor related proteins). Presumably, it is only during exceptional circumstances (*e.g.* the result of a mutation, age-related loss of function or acute stress) that this system of defense is overwhelmed and results in disease. Much remains to be elucidated regarding the role of this system in the development of amyloidosis in the future.

CONCLUDING REMARKS

We here reviewed how the manifestation of protein misfolding diseases is influenced by mutations in disease-causative proteins, interactions of disease-causative proteins with pathological molecular environments, and intracellular and extracellular quality control systems against misfolded proteins. Since the misfolding and aggregation of disease-causative proteins are the

earliest events in the pathogenic cascade, various therapeutic approaches targeting them are extensively being investigated (*8*, *46*). We hope that therapeutic candidates such as protein aggregate inhibitors and chaperone inducers are developed in the near future for the treatment of these currently untreatable protein misfolding diseases.

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CONFLICT OF INTEREST

None declared.

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