Proteostasis and Proteolysis

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Chapter 2

Protein Folding And Misfolding: Deciphering Mechanisms Of Age-Related Diseases

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CHAPTER TWO

Protein Folding and Misfolding: Deciphering Mechanisms of Age-Related Diseases

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2.1 INTRODUCTION

2.1.1 Kinetic and Thermodynamic Aspects of Protein Folding

Protein folding refers to the physical process by which a protein obtains its native state and becomes functionally active. As correct protein folding is a prerequisite for proteostasis in biological systems, determining the mechanisms of this process is crucial for understanding age-related diseases, especially proteinopathies, which are also named proteopathies or protein-folding diseases. A breakthrough in determining three-dimensional structures of proteins occurred in

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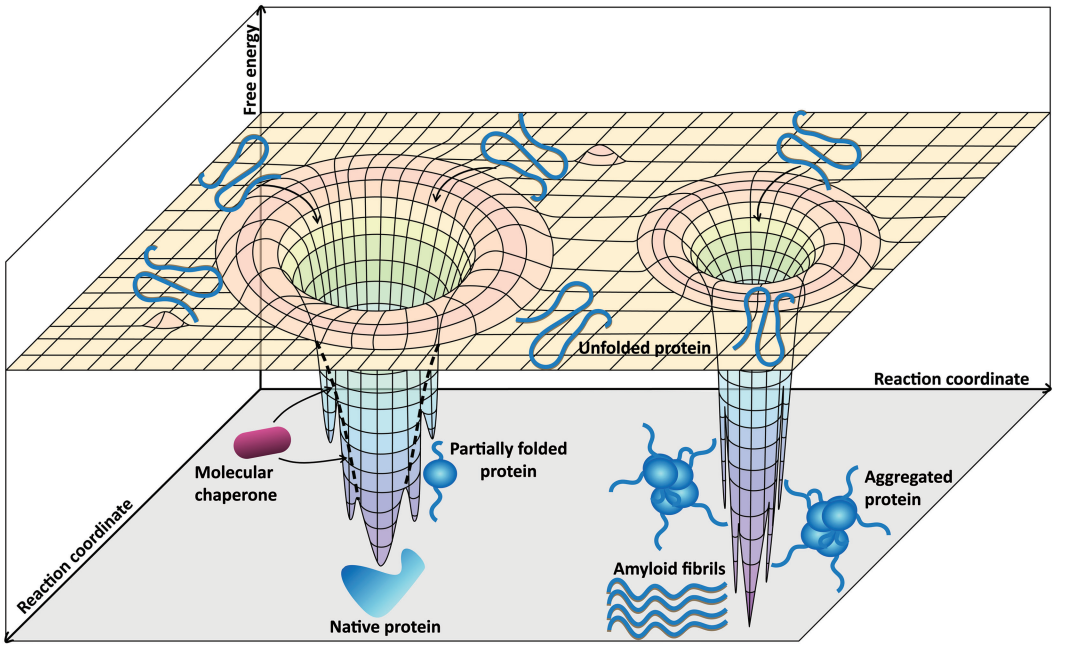

Figure 2.1 **Protein-folding and misfolding energy landscape.** During protein folding, unfolded proteins may have to surpass high free energy stages in order to "fall" into the folding funnel and reach their native conformation, thereby minimizing their free energy level. Molecular chaperones help to prevent trapping of partially folded proteins in low energy states. In case of protein misfolding, aggregated proteins can reach low free energy states and even form highly stable amyloid fibrils with minimal free energy levels.

the 1940s and 1950s, when Kendrew et al. (1958) and Pauling et al. (1951) discovered elements of secondary, tertiary and quaternary protein structures. Then, Anfinsen et al. (1961) demonstrated in 1961 for the small protein ribonuclease A that the information for its folding is inherent in its primary sequence. Another important theorem was given by Levinthal with the "Levinthal's paradox", which states that a protein has an astronomical number of possible conformations due to the high number of degrees of freedom in its chain (Levinthal, 1969). However, according to biophysical measurements, some small simple proteins can be folded in their active form in less than 50 μs, although the number of their possible conformations is extremely high (Mayor et al., 2003). This contradiction was explained by Anfinsen's thermodynamic hypothesis (Anfinsen's dogma) in 1973. It states that the unique native structure that is formed in the environment at which protein folding takes place is the most thermodynamically stable conformation (Anfinsen, 1973). Thus, according to Levinthal's and Anfinsen's hypothesis, many proteins are believed to self-assemble under thermodynamic control (Varela et al., 2019).

In general, the process of protein folding has two stages. Firstly, a molten globule is formed from the random coil of an unfolded protein, losing entropy with little change in energy. In the second, slower stage, the molten globule evolves into the native conformation with reduced free energy and only a small change in entropy (Shirdel and Khalifeh, 2019). These two stages combined form a "folding funnel" (Figure 2.1).

Before a protein reaches its native state, the protein folding goes through a stochastic search of conformations. In this process, the number of possible conformations is irrelevant. Of importance are the kinetically controlled conformations with distinct transition states. In other words, the kinetic control of folding events allows the existence of thermodynamically unstable molecular structures, which is called kinetic trapping (Dill, 1999). In order to prevent kinetic trapping of partially folded proteins, molecular chaperones assist in protein folding (Figure 2.1).

Although most proteins require a defined three-dimensional structure to fulfill their functions, there are some functional proteins in cells that can be either fully unstructured or have only

TABLE 2.1
Frequently used methods to analyze protein folding.

Methods	Physical principles	Output
X-ray crystallography	Scattering of X-ray waves through the atoms' electrons of a protein crystal	Three-dimensional electron density map of electrons within a crystal. Gives information about protein structure
Nuclear magnetic resonance spectroscopy	Atomic nuclei respond to oscillating magnetic fields depending on the local molecular environment	A map of chemical bonds between the protein's atoms and their relative position in three-dimensional protein structure
Cryo-electron microscopy	A beam of electrons is transmitted through a sample cooled to cryogenic temperature	Thousands of pictures taken from various perspectives are combined to form a 3D model
Small-angle X-ray scattering	Scattering of a monochromatic X-ray beam through the structures with 1–100 nm dimensions	Function of electron density distribution within a protein. Gives information about protein shape and size
Circular dichroism	Different absorption of left and right circular polarized light through the chiral systems like proteins	Circular dichroism spectrum that is unique for each conformation of a single protein
Dynamic light scattering	Light scattering through particles like protein molecules	Information about protein size. Changes in molecular size of proteins during folding/unfolding can be observed
Fluorescence spectroscopy	Aromatic amino acids like Trp and Tyr emit light that is absorbed, while emission peaks depend on the polarity of the local environment	A fluorescence spectrum gives information about protein-folding state and conformation

unstructured regions; they are termed intrinsically disordered proteins (IDP) and intrinsically disordered regions (IDR), respectively (Dyson and Wright, 2005). Notably, many proteins involved in proteinopathies contain IDRs, including Aβ, α-synuclein and the pathologic form of prion proteins (PrP^{sc}) (Knowles et al., 2014).

A variety of methods exists that allows to study protein folding and misfolding as summarized in Table 2.1.

2.2 MOLECULAR CHAPERONES

2.2.1 Definition of the Term Molecular Chaperone

Molecular chaperones can be defined as proteins occurring in all taxonomic domains of life that assist other cellular proteins (clients) to adopt their final physiologically active form without being part of their functional structure (Hartl, 1996). Molecular chaperones should be distinguished from chemical chaperones, which are small physiological or non-natural molecules including osmolytes and hydrophobic compounds that can enhance protein stability and folding (Ignatova and Gierasch, 2006). Molecular chaperones do not have a built-in-concept of their clients resembling a catalytic center, which discriminates them from folding catalysts. They are enzymes such as peptidyl-prolyl-isomerases or protein disulfide isomerases, which can break and build covalent bonds (Wang et al., 2015).

2.2.2 Significance of Molecular Chaperones in the Cell

Most molecular chaperones can be upregulated and activated by cells in response to stressful conditions including abnormal temperature and redox states, whereas some molecular chaperones are constitutively expressed. Many, but not all heat shock proteins (Hsp), are molecular chaperones, and *vice versa* many, but not all molecular chaperones, are Hsps (Nollen and Morimoto, 2002). Constitutively expressed molecular chaperones are often abbreviated as Hsc (heat shock cognate proteins). Compared to a simple folding experiment with a single protein in a test tube (Anfinsen

TABLE 2.2

Major molecular chaperone families, their structural characteristics and selected functions.

Molecular chaperone family	Examples prokaryotes	Examples humans	Nomenclature for human genes	Oligomerization degree	Selected functions
Hsp110	–	Hsp110	HSPH	Monomer (interacting with Hsp70)	Recognition of unfolded proteins; nucleotide exchange factors (NEF) for Hsp70
Hsp100	Clp family	Hsp104	–	6-/7-mer	Folding of newly synthesized proteins, disaggregation of aggregated proteins; stress tolerance
Hsp90	HtpG	Hsp90	HSPC	Dimer	Regulation of HSR; signal transduction, e.g. transport of hormone receptors; stabilizing proteins
Hsp70	DnaK	Hsp70, Hsc70	HSPA	Monomer (interacting with Hsp40 and Hsp110)	Binding and folding of nascent chains and unfolded proteins; protein transport; refolding of proteins; heat shock regulation
Chaperonins (Hsp60)	GroEL/ES	Hsp60, TRiC/CCT	HSPE	14-/16-mer	Folding of non-nascent proteins; specialized in their substrates, e.g. TRiC/CCT with actin and tubulin
Hsp40	DnaJ	Hsp40, Hdj	DNAJ	Monomer (interacting with Hsp70)	Substrate holding (holdase); essential co-factor for the ATP-dependent Hsp70 chaperones
Small Hsps	Ipb, Hsp20	Hsp25, Hsp27	HSPB	8- to 24-mer	Protein folding; preventing misfolding and aggregation; microfilament stabilization

et al., 1961), folding of proteins in cells is much more complex making the presence of molecular chaperones inevitable. Firstly, protein folding occurs in a crowded environment, leading to an excluded volume effect. This stems from the fact that macromolecules occupy a large proportion of the available volume in the cell, which reduces the volume of solvent that is available for protein folding. As a result, proteins have fewer degrees of freedom for folding (Ellis, 2001). Secondly, the protein emerges slowly from the ribosome tunnel during translation, and exposed hydrophobic patches may interact with each other intra- or intermolecularly (Etchells and Hartl, 2004). Molecular chaperones protect these exposed regions and prevent misfolding and tagging for degradation. Thirdly, the proteome, especially in eukaryotic cells, is diverse and needs different folding microenvironments, to assist different classes of proteins in folding (Kerner et al., 2005). Especially chaperonins, that are large cylindrical molecular machines, can discharge the clients into their cavity and facilitate folding.

2.2.3 A Network of Molecular Chaperones

Molecular chaperones are grouped into families and named according to their molecular weight (Table 2.2). To ensure that the folding of the proteome in its entirety is covered and the flux through the network of molecular chaperones is maintained, protein folding is often organized in folding pathways. It is therefore widely accepted that one molecular chaperone can hand over its clients to the next molecular chaperone, sometimes belonging to a different family (Figure 2.2). Some molecular chaperone systems are ATP-dependent, including Hsp60, Hsp70 and Hsp90. Chaperonins belong to the Hsp60 family and they are special in that they provide a protected environment as a nanocage for ATP-assisted folding cycles (Hayer-Hartl et al., 2016). Members of

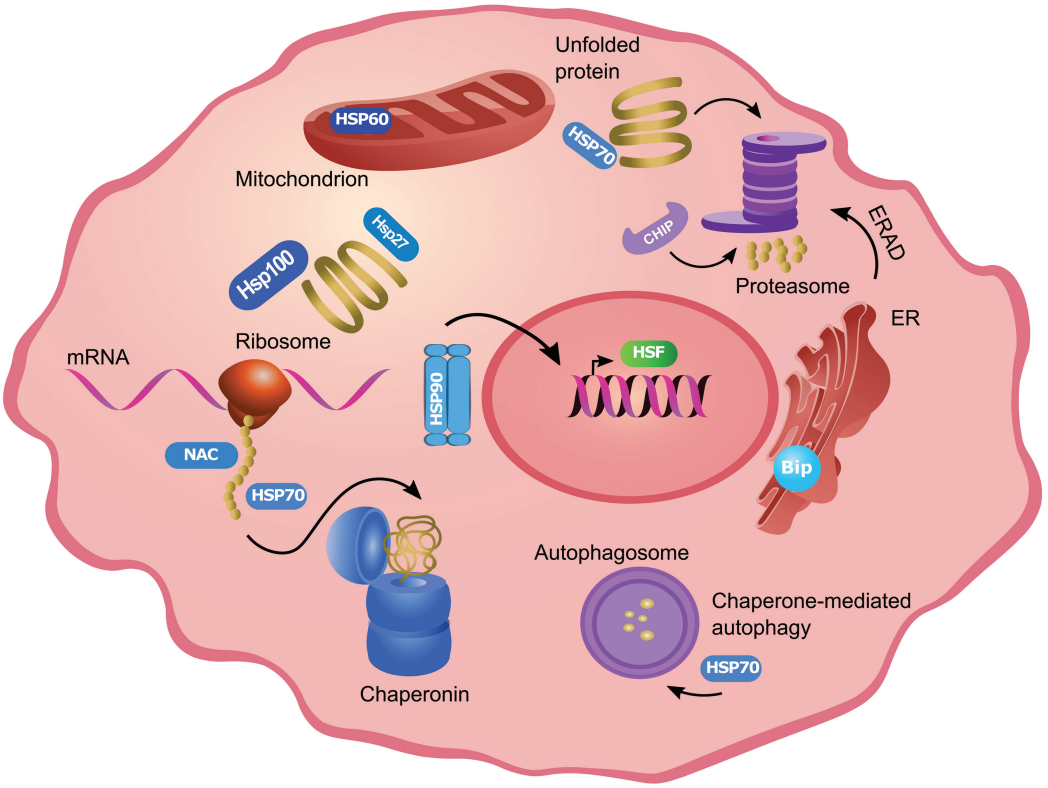

Figure 2.2 **The network of molecular chaperones.** The figure displays a network of the major molecular chaperone families in a eukaryotic cell and interactions with other protein quality control (PQC) systems. Molecular chaperones are depicted in blue, nucleic acids in magenta, protein chains in yellow, transcription factors in green and biomolecular degradation systems in purple.

other Hsp families can simply bind and stabilize the client in an ATP-independent manner such as Hsp40 and some of the small Hsps. The respective chaperones are sometimes termed foldases or holdases, respectively. The concerted action of the molecular chaperones needs to be tightly regulated with the help of heat shock factors (HSFs). HSFs are transcription factors that are bound by molecular chaperones, which can be released upon cellular stress, bind onto special regions of the genome called heat shock response elements (HSE) and initiate the transcription of stress response genes (Sarge et al., 1993).

2.2.4 Functions of Molecular Chaperones and Interplay with Other Protein Quality Control Systems

Aside from assisting in co-translational and post-translational *de novo* protein folding, molecular chaperones can refold misfolded proteins and destroy protein aggregates (Nillegoda et al., 2015). Molecular chaperones do not act only in the cytoplasm; they are important proteostatic factors in the nucleus, endoplasmic reticulum (ER), mitochondria and chloroplasts, where they are also involved in protein transport.

A stress response similar to the heat shock response in the cytoplasm can occur in the ER. The cell handles misfolded proteins in the ER by initiating an unfolded protein response (UPR), where ER-residing chaperones such as Bip (binding immunoglobulin [Ig] protein) help with protein folding but also retrograde transport through the ER membrane (Walter and Ron, 2011). This is tightly coupled with the ER-associated protein degradation (ERAD) (Christianson et al., 2011). During ERAD, misfolded proteins are recognized and transported back into the cytoplasm, where they are handed over to the ubiquitin-proteasome system (UPS). Chaperones binding misfolded proteins in the cytoplasm can be recognized by

adaptor proteins which label the protein with ubiquitin for degradation, like the E3 ligase CHIP (C-terminus of HSC70 interacting protein) (Smith et al., 2013). Some proteasomal shuttling factors belonging to the UbL/UbA (ubiquitin-like/ubiquitin-associated protein) family, such as HR23B, contain chaperone-binding domains and may interact with molecular chaperones (New et al., 2013). Molecular chaperones also play a role in targeting misfolded proteins for autophagy, especially for chaperone-mediated autophagy (CMA) (Kaushik and Cuervo, 2012).

2.3 PROTEIN MISFOLDING

As mentioned above, the free energy of a protein is determined by intramolecular interactions between amino acid residues. Thus, even small changes in the protein chain, for example due to mutations or aging, can cause reshaping of the folding funnel landscape, which may result in the formation of a new global free-energy minimum. This new stable state can initiate protein misfolding and therefore lead to different adverse effects including loss of protein function and aggregation (Clark, 2004).

2.3.1 Process of Protein Misfolding

Most proteins can fold from their native state toward misfolded species, crossing different intermediate states. However, misfolded species and aggregates can originate from both intermediate and native states of a protein (Clark, 2004) (Figure 2.3).

Due to the high-energy barrier that needs to be surpassed to obtain the native conformation, molecules can get trapped in partially folded states. These proteins might interact with other partially folded proteins and form aggregates, as they often expose hydrophobic amino acid residues and domains on their surface (Liu and Eisenberg, 2002). The process of aggregation is concentration-dependent, driven by hydrophobic forces and can result in amorphous or highly organized structures (Chiti and Dobson, 2006). Interestingly, some misfolded proteins are able to convert their native protein variants into misfolded proteins; these types of proteins are called prions (proteinaceous infectious particle derived from the words **pr**otein and infect**ion**) (Prusiner, 1982). Protein aggregation can also be caused by aberrant posttranslational modifications (PTM) including proteolytic cleavage, glycosylation, phosphorylation, ubiquitination or acetylation (Olzscha, 2019; Olzscha et al., 2017).

2.3.1.1 Amyloid Proteins

Protein aggregates can be more stable than native proteins due to the depth of the kinetic trap (Gregersen et al., 2006). Through nucleation processes, aggregates can initiate the formation of pre-amyloid oligomers, which can grow into protofilaments and eventually mature fibrils with cross-β-structures. These structures are characterized by a perpendicular arrangement of β-strands along the fibril axis. Once a certain size of a protein-consisting nucleus is reached ("critical nucleus"), aggregates grow exponentially, due to partial dissociation, providing an increasing number of seeds that repeatedly form new aggregates. In case of amyloid fibrils that are highly organized and stable structures, spontaneous dissociation into monomers is unlikely (Lashuel et al., 2002). Abnormal deposition of such amyloid structures in extracellular compartments can be observed in a wide group of diseases called amyloidoses (Benson et al., 2018).

2.3.2 Factors Leading to Protein Misfolding

2.3.2.1 Mutations

Genetic alterations can cause changes in protein structure, function or localization within the cell. Even minor modifications can make a protein prone to misfold or aggregate which leads to a number of inheritable diseases (Gámez et al., 2018) (Figure 2.4).

For example, a single nucleotide polymorphism can lead to the manifestation of cystic fibrosis (CF), as demonstrated by the phenotypes associated with the ΔF508-*CFTR* (CF transmembrane conductance regulator protein)-mutated allele. This allele carries a deletion of a phenylalanine, leading to the misfolding of CFTR in the ER and its impaired trafficking toward the plasma membrane (Goor et al., 2006).

2.3.2.2 Nongenetic Causes

During aging, PQC systems begin to malfunction, which can lead to the accumulation of unfolded, misfolded and aggregated proteins.

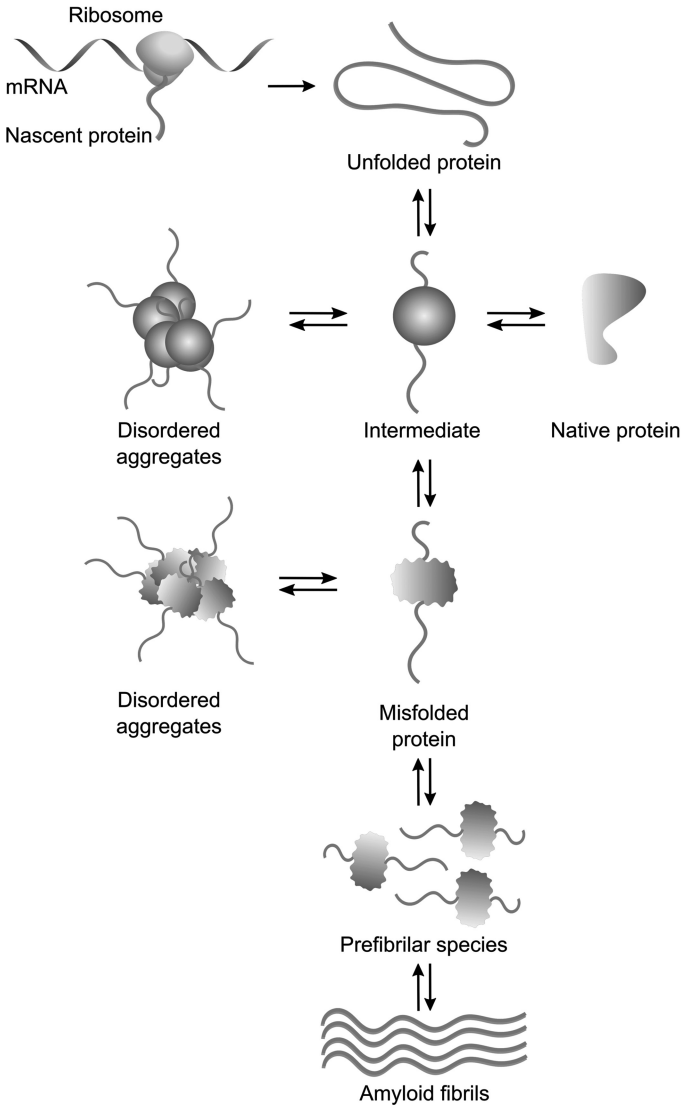

Figure 2.3 **Protein folding and misfolding.** Different steps in protein folding, misfolding and aggregation starting with the nascent polypeptide chain are displayed.

For instance, the heat shock response (HSR) becomes less sensitive with age, leading to slower and reduced activation of molecular chaperones (Calderwood et al., 2009). The increased amount of toxic protein species can then lead to cellular dysfunction, further impairment of the PQC systems and disease acceleration (Gidalevitz et al., 2006) (Figure 2.3).

Like aging and mutations, high levels of stress, including oxidative stress, can cause protein misfolding. High amounts of reactive oxygen species (ROS) in a cell can affect biomolecules and lead to lipid peroxidation as well as a range of different PTMs, which may make proteins prone to misfolding or aggregation (Levy et al., 2019). Aldehyde products resulting from lipid peroxidation such as 4-hydroxy-2-nonenal (HNE) can then further trigger formation of aberrant PTMs and this has been shown to give rise to toxic oligomeric species formed by the affected proteins (Xiang et al., 2015). Neurons in particular tend to be more vulnerable to oxidative stress than other cell types due to several reasons. Firstly, they are highly dependent on glucose oxidation as a major

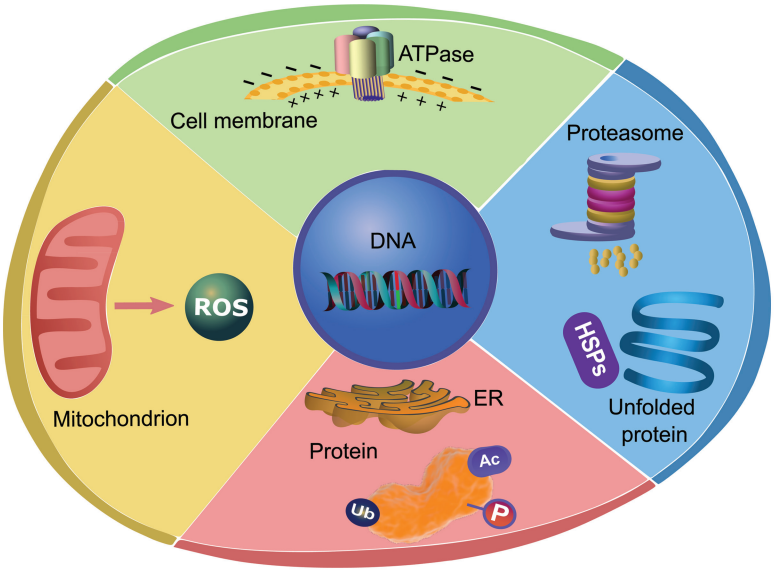

Figure 2.4 **Factors leading to protein misfolding.** There is a variety of factors affecting the protein-folding process and thus leading to misfolded protein species including mutations (dark blue section), chemiosmotic stress (green section), declining protein quality control (light blue section), aberrant posttranslational modifications (red section) and redox reactions (yellow section).

energy source. An imbalance in energy-producing processes, for example through aging mechanisms, can significantly affect redox homeostasis. Secondly, due to their post-mitotic state, neurons become more prone to the accumulation of ROS (Cobley et al., 2018). In Parkinson's disease (PD) for example, mitochondrial dysfunction and increase of intracellular ROS play a key role in pathogenesis (Schapira and Gegg, 2011) (Figure 2.4).

2.4 AGE-RELATED PROTEINOPATHIES

Misfolding and aggregation of proteins can cause cytotoxicity through many different pathways. Considering that these proteins do not reach their native conformation, they cannot execute their normal functions. This can lead to disturbances of cellular processes, cell death and can trigger disease onset.

Moreover, protein misfolding may have harmful effects based on the gain-of-function hypothesis of misfolded proteins. Research also suggests that oligomeric species that precede fibril formation in aggregation processes induce greater cytotoxic effects than mature fibrils and play a key role in driving neurodegeneration (Rockenstein et al., 2014). They characteristically expose hydrophobic domains, singular β-strands and IDRs on their surface, interacting with proteins of various functions, often IDRs themselves and involved in key cellular processes (Olzscha et al., 2011) (see Table 2.3).

2.4.1 Systemic Proteinopathies

Amyloidoses are diseases with various clinical manifestations in which amyloid structures accumulate in tissues. Proteinopathies and amyloidoses can be discriminated, based on their localization, in systemic and localized diseases. One example of a systemic form of amyloidosis is β_2-microglobulin amyloidosis ($A\beta_2M$). High concentrations but also mutations of the protein (hereditary $A\beta_2M$-amyloidosis) can make it prone to misfolding and lead to consequent deposition in different connective tissues (Stoppini and Bellotti, 2015). Clinical signs are carpal-tunnel syndrome and other joint pains as well as bone cysts and consequent pathological fractures.

Multiple myeloma (MM) is a hematologic malignancy in which monoclonal plasma cells in the bone marrow produce high amounts of Igs uncontrollably. In the case of Bence-Jones protein (BJP)-MM, mostly Ig light chains accumulate, which can cause kidney damage (Umberto et al.,

TABLE 2.3

Selected array of age-related conformational diseases.

Proteinopathy	Pathophysiology	Aggregated/ Misfolded protein	Age of onset	Clinical features
		I. Systemic		
Systemic amyloidosis ($A\beta_2M$)	Impaired renal clearance/ dialysis therapy/ misfolding mutations cause accumulation of $A\beta_2M$	β_2-Microglobulin	Usually following chronic kidney disease	Arthralgia, bone cysts and pathological fractures
Amyloid transthyretin amyloidosis (ATTR)	TTR conversion into amyloid fibrils Familial amyloid polyneuropathy (FAP): Val30Met mutation Familial amyloid cardiomyopathy (FAC): Val122Ile mutation Wild-type ATTR amyloidosis (ATTRwt): acquired during aging	Transthyretin	50–60 years ATTRwt: >60 years	FAP: polyneuropathy, neurogenic bladder, autonomic dysfunction FAC: arrhythmia, heart failure ATTRwt: cardiological manifestations, arrhythmia, carpal tunnel syndrome
Multiple myeloma (MM)	Malignant degeneration of plasma cells, producing high amounts of Ig/Ig light chains	Igs/Ig light chains Bence Jones protein (BJP)	50–70 years	B-symptomatology, anemia, bone pains, osteolysis
		IIa. Localized (Non-nervous system related)		
Primary localized cutaneous amyloidosis (PLCA)	Mutations in OSMR or IL31RA, amyloid deposits in skin, mechanisms unknown	IL-31 receptor	Adulthood	Pruritus, skin scratching, discolored skin
Cataract	Loss of transparency of eye lens, aggregates form through accumulation of oxidative stress	γ-Crystalline	>65 years	Blurred/Impaired vision
		IIb. Localized (nervous system related)		
Alzheimer's disease (AD)	Inadequate clearance/ increased production of $A\beta$, hyperphosphorylation of tau	$A\beta$ Tau	>65 years	Dementia, loss of cognitive functions
Frontotemporal dementia (FTD)/ Frontotemporal lobar degeneration (FTLD)	FTLD-tau: astrocytic plaques, corticobasal degeneration, Pick bodies FTLD-TDP: cytoplasmic inclusions, dystrophic neurites	Tau TDP-43	45–64 years "Early-onset dementia"	Dementia, prominent behavioral features, language deficit
Parkinson's disease (PD)	Degeneration of dopaminergic neurons, neuronal inclusions of aggregated α-synuclein	α-Synuclein	50–60 years	Bradykinesia, muscular rigidity, tremor

(*Continued*)

TABLE 2.3 (CONTINUED)

Proteinopathy	Pathophysiology	Aggregated/ Misfolded protein	Age of onset	Clinical features
Lewy body dementia (LBD)	Majority sporadic, rare mutations: SNCA, LRRK2 gene. Neuronal inclusions of α-synuclein, with or without Aβ Cerebrovascular pathology	α-Synuclein Aβ	65 years	Dementia, deficit in attention span, executive function Visual hallucinations, spontaneous parkinsonism
Multiple system atrophy (MSA)	Glial and neuronal inclusions of α-synuclein, demyelination, gliosis	α-Synuclein	55–65 years	MSA-P (parkinsonian): parkinsonism MSA-C(cerebellar): ataxia Autonomic failure, sleep disorders
Spinocerebellar ataxia (SCA)	PolyQ expansions in different proteins, aggregation in cerebellar neurons	Ataxin-1/2/3/7 TBP (TATA-binding protein) CACNA1A (calcium channel)	Adulthood Depending on SCA type	Ataxia, neuronal atrophy, specific symptoms in different SCA forms
Huntington's disease (HD)	Mutation in IT-15 gene PolyQ expansions and aggregation of Htt	Huntingtin (Htt)	30–50 years	Abnormal motor movements, personality change
Prion's disease	Infectious proteinaceous particles (prions) lead to misfolding/aggregation of other proteins	Prion protein	Around 60 years Latent onset (20-30 years after infection)	Psychopathological abnormalities, severe dementia, myoclonus
Amyotrophic Lateral Sclerosis (ALS)	Aberrant modification TDP-43 protein Mutation in SOD1 gene	TDP-43 Cu/Zn superoxide dismutase (SOD1)	Familial: 50 years Other: 60–80 years	Progressive loss of muscular strength, muscle cramps
Familial neurohypophyseal diabetes insipidus (FNDI)	Mutation in AVP gene	Neurophysin II	N/A	Polydipsia, polyuria

2016). BJPs can form aggregates like amyloid fibrils and precipitate in tissues. The underlying mechanisms are not fully elucidated, although some mutations have been found to change the aggregation propensity of the proteins (Timchenko and Timchenko, 2018). Clinical features of MM can encompass fatigue, anemia, skeletal pain as well as osteolysis that results in hypercalcemia. One therapeutic strategy includes administration of proteasome inhibitors, which lead to an abundance of misfolded Ig within the mutated plasma cells due to their high production rate, resulting in ER-stress and subsequent apoptosis (Gandolfi et al., 2017).

2.4.2 Localized Proteinopathies

Proteinopathies can also manifest in specific cell types and therefore affect only distinct organ systems.

2.4.2.1 Non-nervous System-Related Diseases

A prominent example of a localized non-nervous system-related and age-dependent protein-misfolding disease is cataract. The crystalline proteins within the eye lens serve to transmit and focus light into a bundle, in order to be projected onto the retina at the back of the eye. During

aging, different toxic agents accumulate, in particular through UV radiation, oxidation and deamination which make crystalline proteins prone to misfolding and aggregation (Moreau and King, 2012). The protein solution loses its transparency, the lens becomes cloudy and vision is impaired.

2.4.2.2 Nervous System-Related Diseases

An abundance of proteinopathies manifests in the nervous system with increasing age. With little or no ability to regenerate or replace affected cells, the capacity of the nervous system to cope with increasing amounts of misfolded or aggregated species is reached with time leading to disease onset (Cenini et al., 2019).

Alzheimer's disease (AD) is one of the most common age-related neurodegenerative diseases, characterized by progressive cognitive impairment and dementia. Major histological manifestations include senile plaques consisting of pathological forms of the Aβ peptide in the extracellular matrix and misfolded tau species arranged in intracellular neurofibrillary tangles (NFT) (Lane et al., 2018). Aβ is produced through cleavage of membrane-associated amyloid precursor protein (APP) by β- and γ-secretases. An inadequate clearance of the peptide or mutations in either APP or APP cleaving secretase complexes can trigger accumulation and aggregation of Aβ and amyloid plaque formation (Gouras et al., 2015). Oligomeric Aβ peptides have been found to induce neuroinflammation, mitochondrial dysfunction and oxidative damage, as well as influence synaptic efficacy and kinase/phosphatase activities (Lane et al., 2018). In addition, hyperphosphorylation of microtubule-associated tau protein occurs and results in misfolding and the development of NFT (Iqbal et al., 2009).

Another highly prevalent neurodegenerative disorder is PD, which is predominantly characterized by motor deficits including bradykinesia, muscular rigidity and resting tremor. Pathological hallmarks include the degeneration of dopaminergic neurons in the *substantia nigra* and intracellular inclusions called Lewy bodies (LB) and Lewy neurites (LN). These formations consist primarily of misfolded or aggregated α-synuclein, which is a presynaptic protein considered an IDP (Benskey et al., 2016). Mutations in the SNCA

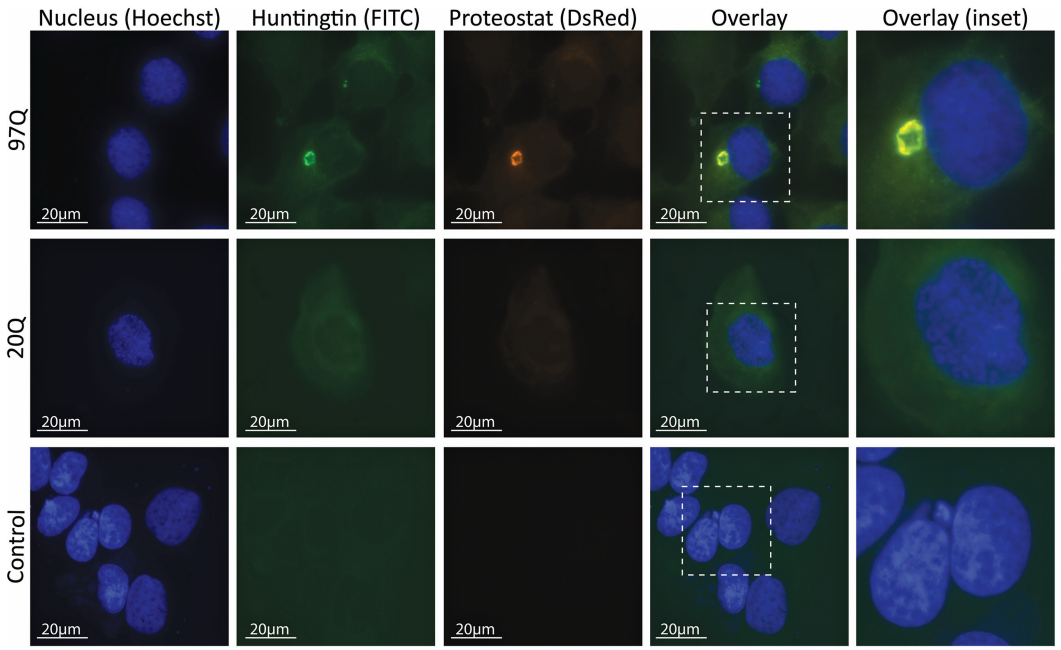

Figure 2.5 **Detection of aggregated Htt in human U2OS cells using epifluorescence microscopy.** Cells were transfected with HA-tagged exon 1 Htt constructs expressing different polyQ stretch lengths. The cells were stained using an HA antibody (green), Hoechst (blue) and Proteostat (red), a dye that binds aggregates. Cells transfected with the 97Q construct displayed Htt aggregates while the 20Q-transfected cells had an even distribution of HA-tagged Htt without aggregates.

gene, which encodes for α-synuclein, lead to a family of diseases called synucleinopathies (Stefanis, 2012). Synucleinopathy-related toxicity may be caused by impairment of the UPS (Kanaan and Manfredsson, 2012), mechanical damage of cellular compartments due to the involvement of α-synuclein in many cellular pathways including synaptic vesicle trafficking and mitochondrial dysfunction (Breydo et al., 2012).

Another late-onset proteinopathy, causing abnormal motor movement, depression, personality change and early death is Huntington's disease (HD). It is the result of a mutation of the *HTT* gene, leading to the extension of CAG repeats and a consequential expanded polyglutamine (polyQ) stretch in the encoded protein huntingtin (Htt). PolyQ expansions are considered pathological at >35 repeats (Shacham et al., 2019) while the CAG-repeat length determines the age of onset that is between 30 and 50 years. The mechanism underlying neurotoxicity in HD is the misfolding and aggregation of mutated huntingtin due to its polyQ expansion (Figure 2.5).

Amyotrophic lateral sclerosis (ALS) is one of the most common motor neuron diseases in adults. A determining clinical feature is the progressive muscle weakness leading to loss of motor movement and inevitably to respiratory failure (Scotter et al., 2015). The TART-DNA binding protein 43 (TDP-43) shows aberrant modifications in 97% of ALS cases and can be found aggregated in ubiquitinated inclusions in the brain and spinal cord of ALS, as well as frontotemporal lobar degeneration (FLD) patients (Prasad et al., 2019). In some cases, the superoxide dismutase-1 (SOD1) gene is mutated and leads to SOD1 accumulation in inclusions. Although there is no definite data on how TDP-43 or SOD1 might affect neurotoxicity in ALS, it is suggested that a combination of toxic gain and loss of function might be crucial (Mackenzie et al., 2007).

Prion diseases represent a group of neurodegenerative diseases where protein-only infectious agents cause fatal brain damage. PrP^{Sc} are misfolded species that form through conformational changes of the normally folded protein PrP^{C} (Collinge, 2016). PrP^{Sc} forms perineural plaques, causing a typical pathological pattern, also known as spongiform encephalopathy. This process is amplified, because PrP^{Sc} can cause misfolding and conformational changes in PrP^{C} and eventually in other proteins. This so-called templating plays a key role in the prion hypothesis, which suggests that the same processes could underlie the pathological mechanisms of other neurodegenerative diseases (Soto and Pritzkow, 2018). Specifically, it has been hypothesized that misfolded proteins can propagate from cell to cell and initiate templating in neighboring cells (Guo and Lee, 2011).

2.5 CURRENT AND FUTURE TREATMENTS

Although there have been many discoveries in the field of age-related proteinopathies, development of efficient therapy strategies has not been entirely successful. Nonetheless, there have been several approaches particularly focusing on modulating PQC systems because of their major role in disease development during aging (Kulka et al., 2020). It is important to note that not only reduction of protein aggregation but also management of toxic oligomeric species should be the target of a therapy (see Table 2.4).

Proteostasis regulators (PRs) are molecules that can interfere with PQC functions and signaling pathways. They can modulate cellular response mechanisms like the cytoplasmic HSR and ER-associated UPR through a number of different mechanisms (Muntau et al., 2014).

Modulation of HSFs has been shown to reduce levels of aggregated proteins and restore proteostasis in several disease models. Non-steroidal anti-inflammatory drugs (NSAIDs), for example, can induce HSF-1 or ensure complete activation of HSR under stress conditions (Jurivich et al., 1992).

Another class of PRs is Hsp90 inhibitors like geldanamycin and its derivatives, which are presently used in cancer therapy. Shifting the conformation of Hsp90 from an ATP-binding to an ADP-binding complex, its chaperone function is inhibited and its association with the UPS is enabled. This leads to disassociation and degradation of Hsp90 client proteins, including steroid receptors, tyrosine kinases and HSF-1, which can initiate the HSR (Sittler et al., 2001).

Proteins that act as sensors of misfolded/unfolded species and conduct the UPR^{ER} are the inositol-requiring protein 1 (IRE1), the translation factor 6 (ATF6) and the protein kinase RNA (PKR)-like ER kinase (PERK). ATF6 activates genes involved in protein folding (i.e. ER-associated chaperones), PERK inhibits mRNA translation and IRE-1 upregulates the ERAD pathway and ER-associated chaperones via the X-box-protein 1

TABLE 2.4
Novel therapeutics in clinical trials affecting protein-misfolding pathologies.

Drug category	Drug/Substance	Phase	Mode of action	Application	References
HSP inhibitors	1) XL888 2) Vemurafenib	I	1) Hsp90 inhibitor 2) BRAF kinase inhibitor	BRAF-mutated stage III/IV melanoma	NCT01657591
	Onalespib	I	Hsp90 inhibitor	Advanced triple negative breast cancer	NCT02474173
	PU-AD	II	Small molecule epichaperome inhibitor	ALS AD	NCT04505358 NCT04311515
Proteasome inhibitors	Ixazomib	I/II	Proteasome inhibitor	Light chain amyloidosis	NCT03236792
Proteostasis regulators	AMX0035	II	Combination: tauroursodeoxycholic acid (TUDCA): inhibits ER/mitochondrial stress-mediated apoptosis, reduces formation of ROS Sodium phenylbutyrate (PB): chemical chaperone, histone deacetylase inhibitor	ALS	NCT03488524
NSAID	ALZT-OP1	III	Combination: ibuprofen (NSAID) Cromolyn (mast cell stabilizer)	AD	NCT02547818
ASO	RG6042	I	Antisense oligonucleotide (ASO), reduces concentration of Htt mRNA	HD	NCT04000594
Small kinetic stabilizer	AG10	III	Transthyretin stabilizer	ATTR – cardiomyopathy Transthyretin amyloid polyneuropathy (ATTR-PN)	NCT03860935 NCT04418024
Antibodies	Lecanemab (BAN2401)	III	Monoclonal anti-Aβ protofibril antibody	AD	NCT04468659
	PRX004	I	Monoclonal anti-amyloid transthyretin antibody	ATTR	NCT03336580

(Ron and Walter, 2007). Small molecule IRE-1 inhibitors have been discovered to have cytotoxic effects in hematological pathologies. STF-083010, for example, inhibits IRE-1 endonuclease activity and increases the inherent ER stress in MM cells, which translates into cytotoxicity (Papandreou et al., 2011).

Complementing the aforementioned approaches, chemical chaperones can aid to sustain protein stability by broadening the free energy gap between the partially folded and native states of a protein, therefore decreasing the number of unfolded species of aggregation-prone proteins (Hekmatimoghaddam et al., 2017). Additionally, pharmacological chaperones are specific small molecules that can bind to proteins via van der Waals or electrostatic forces and through hydrogen bonds and can facilitate protein folding and trafficking (Beerepoot et al., 2017).

2.6 CONCLUSIONS

Protein folding is an essential process in virtually all living systems. It has become clear over the past decade that aging has an adverse effect on protein folding toward the native structure and the capacity of PQC systems, including the system of molecular chaperones. One hypothesis suggests that the decline of crucial PQC components leads to the occurrence of misfolded cellular

proteins. Therefore, protein-misfolding diseases are likely to be increasing in aging societies. There are many attempts to cure these age-related diseases; however, most of them have failed so far. It is conceivable that the critical time point for disease intervention has already passed when protein misfolding and aggregation occur, and an upregulation of the capacity of PQC systems has to take place far earlier. That means genetic testing has to be increased and the likelihood of a disease onset needs to be determined. Besides ethical considerations, stratification of patients and assessing the individual risk score for a disease needs the establishment of reliable biomarkers or even molecular signatures to initiate personalized treatment.

AUTHOR CONTRIBUTIONS

Conceptualization, H.O.; writing – original draft preparation, J.-E. R., D.P. and H.O.; writing – review and editing, J.-E. R., D.P. and H.O; visualization J.-E. R. and D.P.; supervision, H.O.; project administration, H.O.; funding acquisition, H.O. All authors have read and agreed to the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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