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Mechanisms of protein homeostasis in health, aging and disease

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Summary

When emerging from the ribosomes, new polypeptides need to fold properly, eventually translocate, and then assemble into stable, yet functionally flexible complexes. During their lifetime, native proteins are often exposed to stresses that can partially unfold and convert them into stably misfolded and aggregated species, which can in turn cause cellular damage and propagate to other cells. In animal cells, especially in aged neurons, toxic aggregates may accumulate, induce cell death and lead to tissue degeneration via different mechanisms, such as apoptosis as in Parkinson's and Alzheimer's diseases and aging in general. The main cellular mechanisms effectively controlling protein homeostasis in youth and healthy adulthood are: (1) the molecular chaperones, acting as aggregate unfolding and refolding enzymes, (2) the chaperone-gated proteases, acting as aggregate unfolding and degrading enzymes, (3) the aggresomes, acting as aggregate compacting machineries, and (4) the autophagosomes, acting as aggregate degrading organelles. For unclear reasons, these cellular defences become gradually incapacitated with age, leading to the onset of degenerative diseases. Understanding these mechanisms and the reasons for their incapacitation in late adulthood is key to the design of new therapies against the progression of aging, degenerative diseases and cancers.

Key words: *HSP70; molecular chaperones; proteasome; aggresome; lysosomal autophagy; amyloid; Alzheimer; Parkinson; cancer*

Cellular youth, immortality, aging and death

This review examines the crossroad between protein homeostasis, aging and the onset of degenerative diseases associated with protein aggregation. It addresses the possible links between aging and the efficacy of different protein folding and degradation machineries that can repair or eliminate toxic misfolded proteins in healthy young cells and also in immortalised cancer cells, but for unclear reasons become defective in old cells, leading to cell death and diseases.

A global snapshot analysis of the Earth's biosphere would show that the overwhelming majority of living cells are neither dividing nor growing. Environmental resources are generally limiting and, consequently, only a minority of organisms may occasionally undergo exponential growth. While being very much alive, the remaining organisms maintain a quiescent renewable life style, in which only minimal energy-consuming tasks are being carried out to maintain steep ion gradients across membranes and, as time passes, to replace stress-worn damaged macromolecules. Life, however, can be exposed to sharp variations of environmental conditions, especially under terrestrial continental climates, far from the buffering effects of large bodies of water. Lipids, polypeptides and, to a lesser extent, polynucleotides and polysaccharides, are sensitive to swift changes in abiotic environmental cues, such as heat, cold, dehydration, ultraviolet light, the formation of reactive oxygen species, etc. Under heat stress, for example, the hydrophobic cores of organic macropolymers, such as membranes and soluble protein complexes, may transiently melt and form instead stable misfolded structures, which are biologically inactive [1].

The process of cellular aging tightly correlates with a general decrease in the efficiency of various cellular repair mechanisms to prevent damage, repair or replace damaged macromolecules, as they form with time, under various stresses. Yet, even without stress, unicellular organisms, such as budding yeast, may undergo age-associated changes, ultimately leading to cell death [2]. Hence, with increasing budding events, the protein homeostasis machineries of the mother cells undergo a decline of quality. They gradually become defective at preventing the toxic aggregation of proteins and at processing damaged structures in proteins [3]. Further, they show modifications and substantial variations of cellular and vacuolar morphologies on their path to death, and they gradually fail to degrade and replace chemically damaged proteins and polynucleotides [4–6].

Aging may not only result from the functional decline of the cellular protein repair and degradation machineries. An age-dependent decline of DNA repair enzymes can also produce mutations, such as double strand breaks, that can directly trigger apoptotic signals [7] or, less directly, unleash carcinogenesis or induce the formation of cytotox-

ic misfolded protein aggregates, leading to the onset of progressive degenerative diseases and death. Impressively, sequencing of individual neuron nuclei from a 15-year-old human brain revealed that over 1500 new single somatic mutations took place during brain development and differentiation. The mutations were predominantly in coding regions of genes involved in nervous system development and mature neuronal function, suggesting a "use it and lose it" scenario, in which the very genes that were used for the function of a neuron were those that became most damaged during the short lifetime of that neuron lineage [8]. It is thus not unlikely that new mutations in various differentiating neuron lineages that would render proteins more aggregation-prone could sooner or later overwhelm the neuron's defences against cytotoxic aggregates and prompt apoptosis and tissue loss, and cause degenerative diseases.

In lower vertebrates such as the salamander, tissue regeneration depends on the ability of differentiated adult cells to undergo reprogramming and become pluripotent again, to regenerate whole amputated limbs [9]. Similarly, when fully differentiated, growth-arrested plant cells are artificially disconnected from their original tissue, some cells may spontaneously revert to being undifferentiated totipotent stems cells, which, in the presence of appropriate hormones, can regenerate into whole plant clones [10]. Adult mammals, however, are composed mostly of terminally differentiated cells that cannot de-differentiate into stem cells and are therefore mortal. Only few pluripotent stem cells are maintained, for example the germline cells, which are immortal by virtue of their optimal capacities to repair stress-damaged macromolecules or eliminate and promptly replenish them [6, 11].

The process by which cells can control the rate of synthesis, folding and assembly of polypeptides into functional proteins and maintain their activity under various stresses for a well-defined time before they must become degraded by controlled proteases and be replaced by new polypeptides, is called protein homeostasis, or proteostasis [12]. Evidence that terminally differentiated cells, rather than becoming simply defective in protein homeostasis, are being actively repressed from being effective in protein homeostasis, comes from a *Caenorhabditis elegans* model for age-dependent toxic polyglutamine aggregation. Mutant nematodes that have arrested germline cells or that are devoid of oocytes or sperm cells accumulate toxic protein aggregates and develop the disease much later and live significantly longer than fertile nematodes [13, 14]. This indicates that an inhibitory signal, probably of hormonal nature, is being sent from the germline cells, which are immortal, to repress the protein homeostasis mechanisms of the nonreproductive cells, which consequently become mortal [13, 15].

Protein misfolding and cellular mechanisms to maintain protein homeostasis

When a soluble protein is rendered labile by mutations and is exposed to various stresses, it may transiently unfold and uncommonly expose hydrophobic residues to the hydrophilic surroundings (fig. 1). It is then thermodynamically compelled to seek an alternatively stable misfolded conformation lacking biological activity, which is generally enriched in non-native β-sheets with partially exposed hydrophobic surfaces [16, 17]. Depending on their concentration, several stress-misfolded polypeptides may further generate intermolecular associations, leading to stable insoluble high-order oligomers, commonly termed aggregates. Small soluble aggregates have been reported to alter membrane integrity, as a result of their exposed hydrophobic residues, by different mechanisms such as the spontaneous assembly of pore-forming annular oligomers [18, 19] or direct damaging punctures by the actively growing amyloid fibrils. Ensuing plasma membrane leakages and mitochondrial depolarisations lead to the aberrant activation of proapoptotic pathways [20, 21].

Amyloid fibrils and tangles are formed by normally soluble proteins, which misfold and assemble to form insoluble fibres that are resistant to degradation. Moreover, in the cell, the disordered aggregates and the more compact protofibrils and tangles can interfere with the cytoskeleton, enhance oxidative stress and, importantly, impair the protein repair and clearance mechanisms [22]. Furthermore, protein aggregates can lure other labile native proteins to misfold and aggregate as well. As with microcrystal seeds inducing the formation of large protein crystals, minute amounts of preformed aggregated proteins can act as seeds (which may be formed by the physical disruption of large aggregates, such as amyloid fibrils) catalysing the forma-

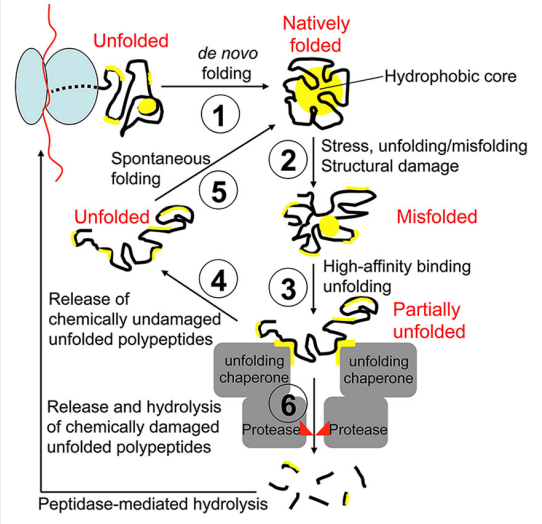

Figure 1

Proteins have an eventful life between birth on the ribosome and death by degradation. Nascent polypeptides with exposed hydrophobic residues (yellow) may spontaneously fold to the native state (step 1) with most hydrophobic residues forming a stable hydrophobic core. Stresses may cause their partial unfolding and misfolding with exposed hydrophobic residues (step 2) that can specifically bind (step 3) the hydrophobic surfaces (yellow) on unfolding chaperones (deep grey) and unfold. Following unfolding, some polypeptides can be released (step 4) to spontaneously refold to the native state (step 5). Alternatively, over-sticky unfolded polypeptides can be funnelled into the proteolytic chamber (red triangles) of a protease that is associated with the chaperone (step 6) and degraded into small peptides and by peptidases into amino acids to be reused in *de novo* protein synthesis.

tion first of disordered, then of increasingly ordered compact protein aggregates and fibrils. This prion-like behaviour may happen not only in the case of the infective agent responsible for transmissible spongiform encephalopathy, PrPsc [23], but with other metastable protein species, such as α-synuclein in Parkinson's disease [24, 25], tau and amyloid-β (Aβ) peptides in Alzheimer's diseases [26, 27], that naturally maintain an equilibrium between natively unfolded, partially unfolded and misfolded states [28]. Although Parkinson's and Alzheimer's diseases are multifactorial conditions, the presence in specific neural tissues of diffuse aggregates that may further evolve into being very stable amyloids and highly ordered fibres in and outside cells is a hallmark of these degenerative diseases. Moreover, the artificial overexpression of specific aggregation-prone proteins can, in part, recapitulate the symptoms of these human diseases in various animal models [29, 30].

There is strong evidence that the soluble disordered aggregated oligomers of the neurodegeneration-associated proteins, which are among the earliest intermediates of protein misfolding pathways, are also the most toxic species for the cells. Thus, Aβ dimers can impair synaptic activity more efficiently than larger complexes [31] and small PrPsc complexes can infect naïve mice more efficiently than large PrPsc fibrils [32]. Moreover, studies in model organisms showed that the formation of high molecular weight aggregates can reduce toxicity, suggesting that active condensation of the disordered aggregates is an effective mechanism to reduce toxicity [33].

Noticeably, under various stresses and because of heavy metal poisoning, small soluble aggregated seeds can form and further promote the aggregation of other proteins. Even substoichiometric amounts of heavy metal ions, which can directly cause the misfolding of only a few polypeptides can, by way of prion-like seed-induced aggregation, accelerate the aggregation of a large molar excess of labile proteins, although most of them cannot physically interact with the traces amounts of toxic heavy metal ions [34]. Hence, the presence of sub-stoichiometric amounts of aggregated seeds can affect equilibria in various populations of stress-labile native proteins, luring them to become stable toxic aggregates [35]. The intracellular prionoid behaviour of such misfolded proteins, which escaped cellular quality control, may, upon cell lysis, propagate to neighbouring cells and tissues [36].

At the physiological level, the stress- and mutation-induced formation and the possible propagation of various misfolded protein conformers in animal cells are closely associated with the formation of reactive oxygen species (ROS), which in animals is in turn known to trigger apoptosis, tissue inflammation and, ultimately, cell death and tissue loss [37–39]. Until the end of the 20th century, ROS were thought to be the direct cause of cellular damage, leading to cell death and tissue loss in aging animals, and of the late-age onset of degenerative diseases [40]. But recent findings indicate that the mutual relations between ROS and viability are more complex than initially thought. On the one hand, low levels of ROS can extend the lifespan of nematodes [41]. On the other hand, experimental evidence from suppressor mutations of ROS-producing *Arabidopsis* plant mutants showed that very high ROS levels are not cytotoxic [42]. Likewise, whereas the deletion of all five superoxide dismutase-encoding genes of *C. elegans* sensitised the nematodes to oxidative stress, it did not affect their lifespan [43]. Although a systematic analysis of ROS-mediated apoptotic signals has not been performed in all cases where toxic protein aggregates are the apparent cause for programmed cell death and tissue loss, it is tempting to speculate that in eukaryotes, ROS could act as general mediators of inexplicably counterproductive apoptotic signals, leading to protein aggregation-induced cell death [44].

Whether all ROS-mediated or not, Aβ aggregation outside, and tau aggregation inside neuronal cells strongly correlate with Alzheimer's disease; huntingtin aggregation with Huntington's disease; α-synuclein aggregation with Parkinson's disease; ataxin aggregation with amyotrophic lateral sclerosis [45] and PrPsc aggregation with Creutzfeldt-Jakob disease [23].

Although all aggregated proteins tested seem to carry some degree of prionoid properties, catastrophic infectious self-feeding protein aggregations seldom occur in young cells, with the noticeable exception of PrPsc prions that may cause childhood death from kuru disease within months of ingestion [46]. This relative resistance of young naive cells and of old immortalised cancer cells to the cytotoxic accumulation of misfolded proteins can be attributed to effective mechanisms to suppress ROS, such as increased levels of ascorbate peroxidase, and to protective carotenoids, as well as to the presence of a very effective network of molecular chaperones, which can accumulate to form up to 10% of the total protein mass of cancer cells [47]. Many conserved families of molecular chaperones, HSP110, HSP100, HSP70, HSP60 and possibly also HSP90, share the ability to recognise and bind with high affinity to exposed hydrophobic patches on the surfaces of misfolded and aggregated proteins. They can use the energy of binding and also of adenosine triphosphate (ATP) hydrolysis to forcefully unfold stable misfolded secondary structures such as wrong β-sheets [48–51], thereby converting them into low-affinity native proteins that do not oddly expose hydrophobic residues.

The first molecular chaperones were discovered in a screen for *Escherichia coli* mutants that failed to assemble phage heads and tails [52]. Bacteria with missense mutations in the genes encoding for GroES, GroEL, DnaK, DnaJ, and GrpE failed to develop mature T4 and lambda phages, a phenotype that was systematically accompanied by an increased sensitivity to mild heat-shock temperatures. Even at a permissive temperature, the mutants had impaired antibiotic resistance and they accumulated insoluble protein aggregates [53, 54, 55], suggesting that the chaperone network could easily become overwhelmed by a stress, such as viral infection or mild heat shock. The chaperone network of eukaryotic cells can also become overwhelmed, for example in rapidly growing tissues or in specialised tissues that need to handle properly the massive and timely synthesis and assembly of particular protein complexes, such actin and tubulin complexes in sperm cells [56], or actin and myosin complexes in muscle sarcomeres [57]. Thus, different healthy and cancerous mammalian tissues may contain quite different levels of various types of chaper-

ones, totalling 5% of the entire protein mass in naive rat hepatocytes (Finka and Goloubinoff, unpublished results), up to 10% in HeLa cells [47], in correlation with their ability to withstand many genetic aberrations and survive treatments with abiotic stresses, radiation and chemotherapeutic drugs [42, 58]. It is therefore not surprising that many chaperones are also heat-inducible and are generally, although inadequately, called heat-shock proteins (HSPs). Under a mild feverish condition of 4 h at 41 °C, human culture cells (Jurkat) may accumulate up to 4% of their total mass in the form of new HSPs, half of which are the three core cytoplasmic chaperones HSC70 (HSPA8), HSP90AA1 and HSP90AB1. This occurs at the cost of an across-the-board mild degradation of hundreds of housekeeping enzymes, such as glyceraldehyde-3-phosphate dehydrogenase, and structural proteins, such as the ribosomal proteins [59].

By virtue of their ability to prevent protein aggregation during stress, and to unfold and disaggregate structurally damaged proteins after stress, the various members of the chaperone network can maintain optimal cellular protein homeostasis, at least in youth [45]. Notably, whereas *ex-vivo* naïve mammalian cells expressing relatively low chaperone levels die after several cell divisions, immortal cancer cells in culture express high chaperone levels [47, 60, 61]. This correlation suggests that the abnormal isothermal constitutive upregulation of specific chaperones in cancer cells might arrest programmed cell death. This was demonstrated in the case of HSP70 (HSPA1A), which is virtually absent in unstressed normal cells but strongly accumulates under various stresses [59], including heat and treatments with chemotherapy drugs [58]. At variance with the inducible HSP70, which can specifically block apoptosis; HSC70 (HSPA8), which shares 90% homology with HSP70, cannot block apoptosis. It is constitutively expressed at very high levels, about 0.5–1% of the total protein mass of nonstressed mammalian cells [59]. It can act both on stress-misfolded proteins and on native substrates, such as clathrin cages to be recycled into free triskelions [62]. It is important to note that in some cases the over expression of specific folding enzymes, such as peptidyl-prolyl cis-trans isomerases, could be deleterious. For example, the deletion of the HSP90 co-chaperone FKBP51, was shown to protect model mice from the toxicity of tau aggregation, and that over-expression of FKBP51 can prevent tau degradation by the proteasome, thereby promoting the conversion of tau into cytotoxic tangles [63].

Despite these very large amounts of chaperones, there are physiological and stressful situations where the chaperone network can become limiting. Hence, when a thermo-sensitive aggregation-prone mutant protein is expressed at a permissive low temperature, it may fold and function correctly in *C. elegans* muscle cells. Yet when a second aggregation-prone protein with a polyglutamine tract exceeding 35 glutamines is co-expressed, the first mutant protein can become inactive and aggregate even at low permissive temperatures, indicating that the chaperone network is overwhelmed by the expressed polyQ protein [64]. An increase in the aggregate load of the cell can also be attained by specific proteasome inhibitors, or by applying a stress, or by the formation of prion-like misfolding species that propagate within and among cells [65, 66].

The hierarchy of the protein homeostasis mechanisms in the cell

At the ribosomal exit, *de novo* synthesised polypeptides first encounter the highly crowded cellular environment, ranging from 100 mg/ml to 350 mg/ml of proteins for the cytosol of eukaryotes and bacteria, respectively. High protein crowding may increase the propensity of the emerging unfolded polypeptides to form wrong secondary structures, in particular aggregation-prone β-sheets with hydrophobic residues abnormally exposed to the hydrophilic cytosol [67]. Trigger factor and DnaK in the case of bacteria, and heat-shock cognate (HSC)70, zuotin and HSPA14 in the case of eukaryotes, are molecular chaperones that can become transiently anchored to the ribosome exits, in the expectation of binding exposed emerging hydrophobic segments [67, 68]. Upon binding, the chaperones may prevent misfolding, actively unfold [69] and, upon dissociation, promote the proper folding of an emerging polypeptide into its native state, in the structure of which most of the hydrophobic residues (fig. 1, yellow) are properly internalised (fig. 1 step 1). A stress, such as heat shock, may partially melt the hydrophobic core of native proteins (fig. 1 step 2) and lead to the formation metastable misfolded structures, which are enriched with wrong β-sheets that expose some hydrophobic residues to water and lead to the spontaneous assembly of oligomeric ensembles of misfolded polypeptide chains, generally called aggregates. In the cell, however, the exposed non-native hydrophobic surfaces on the misfolded polypeptide species can bind with high affinity to specific exposed hydrophobic surfaces at the surfaces of molecular chaperones, such as the upper inner rim of the chaperonin cavities of chaperonin containing t-complex protein 1 (CCT) and GroEL, or on the specific protein-binding sites in the protein-binding domains of HSP40 and HSP70 [70]. Mere hydrophobic binding of metastable non-native misfolded species to the stable native chaperone molecule surfaces may already cause some local unfolding of the misfolded polypeptide substrate (fig. 1 step 3). Concerted ATP-fuelled motion of domains within the chaperones may further pull apart and unfold bound misfolded segments in between or distal to the chaperone binding segments, thereby allowing longer segments of the newly unfolded polypeptide to seek more native-like conformations, ultimately leading to dissociation from the chaperone (fig. 1 step 4) and native refolding in solution (fig. 1 step 5). If the chaperone-bound partially-unfolded polypeptide fails to dissociate within a biologically relevant time, ATP hydrolysis can be recruited to forcefully change the hydrophobic nature of the over-sticky chaperone binding sites into hydrophilic, as in the case of GroEL and CCT [71], thereby forcefully evicting the over-sticky unfolded intermediates and allowing them to fold to the native state. Thus, chaperones can act as polypeptide-unfolding catalysts accelerating the conversion of high-affinity metastable misfolded substrates, such as toxic protofibrils, or alternatively folded substrates such as clathrin cages [62], into stable, low-affinity native products [50]. However, mutations or various external stresses can cause irreversible damage to polypeptides, such as early stop codons, or chemical damage, such as glycation, oxidation

or the hydrolysis of peptide bonds. In such cases, unfolding chaperones cannot lead to the native state.

To avoid chaperone stalling by over-sticky intermediates, different evolutional variants of unfolding chaperones are found in association with a specific protease into which irrecoverably damaged polypeptides can be funnelled to be degraded into small peptides (fig. 1 step 6). For example, ClpA is an ATP-fuelled cylindrical hexameric unfolding/disaggregating ring chaperone forming a complex with ClpP, which is a cylindrical heptameric protease. The ClpA-unfolded polypeptides may thus be unidirectionally funnelled into the ClpP chamber where they are specifically hydrolysed. ClpX and HslU are other ClpA-related unfolding chaperones that also form noncovalent complexes with specific ring-shaped proteases, ClpP and HslV, respectively. Different unfoldase-protease associations are observed in the case of Lon and FtsH, which are both hexameric ATPase cylinders, where each polypeptide has an N-terminal domain acting as the ATP-fuelled unfolding/disaggregating chaperone and a C-terminal domain acting as a chaperone-gated degrading protease ring [72] (fig. 1 step 6). The peptides that are released from these proteases may in turn be further hydrolysed by specific peptidases such as the tricorn aminopeptidases [73] into free reusable amino acids for resynthesis of new unaltered polypeptides. This latter unfolding→degradation→resynthesis pathway (fig. 1 steps 3, 6, 1) is estimated to be at least three orders of magnitude more costly, in terms of hydrolysed ATP molecules, than the first unfolding→refolding pathway (fig. 1 step 3–5) [48]. Interestingly, sequence-wise and structurally, ClpA has a very close homologue called ClpB (HSP104 in yeast and HSP101 in plants), which does not form a complex with a protease. Instead, ClpB acts in close collaboration with another unfolding chaperone, HSP70 (DnaK), through a special M-domain. Together they drive the unfolding and disaggregation of large stable proteins aggregates into native proteins [74–77]. It is tempting to speculate that the evolutionary scenario was that of an initial unfolding chaperone-protease carrying both refolding and degrading functions, which later specialised into the ClpA-ClpP unfoldase-protease and the ClpB-DnaK unfoldase-refoldase. These two diverging activities have been observed in complexes of polypeptides with two distinct segments: an N-terminal ATP-dependent unfoldase domain followed by a C-terminal protease domain. Interestingly, the prokaryotic and organellar AAA+ (ATPase associated with diverse cellular activities) protein Lon can process bound substrates at markedly varying rates; under some conditions, it may function as an ATP-dependent chaperone that mostly refolds misfolded proteins and under other conditions, as an ATP-independent protease that mostly degrades misfolded proteins [72, 78]. A similar duality has been observed in DegP proteins, which may function as both ATP-independent chaperones that mostly refold misfolded proteins and, under other conditions, as a protease that mostly degrades misfolded proteins [79]. The decision between protein repair and recycling could depend, for example, on the time spent by the polypeptide on chaperone surface. Whereas a short stay would increase the chances for the released polypeptide to refold in solution to the native state, a longer stay would increase its chances to become translocated and hydrolysed by the nearby proteolytic sites (fig. 1 step 6, red triangles).

When over-sticky misfolded polypeptides resist unfolding and hydrolysis, they act as competitive inhibitors of the chaperone and/or proteases to which they are bound, preventing the progressive conversion of other misfolded polypeptides. Chaperone-mediated autophagy in eukaryotic cells happens when the stalling polypeptides containing a specific N-terminal lysosomal targeting signal docks the whole substrate+chaperone/protease complex onto lysosome-associated membrane protein 2 (LAMP-2A) [80]. Substrate binding triggers the formation of LAMP-2A multimers that act as a translocation complex to the lysosomes. Whereas the translocation would liberate the stalled cytoplasmic chaperone complex to act again as an unfoldase (fig. 2 step 1), the formerly over-sticky chaperone-resistant polypeptides would be specifically delivered and degraded by the lysosomal proteases [81]. Other chaperone-stalled misfolded proteins, especially those lacking the KFERQ pentapeptide, can form large compact detergent-insoluble aggresomes by transporting the misfolded proteins, most of which are ubiquitinated, along microtubules to a region near the nucleus [82]. Aggresome formation is accompanied by a redistribution of the intermediate filament of vimentin to form a cage surrounding a pericentriolar core of the aggregated proteins (fig. 2 step 2) [83]. Whereas the ubiquitinated polypeptides may then be degraded by the proteasome (fig. 2 step 3), there is still a possibility for nonubiquitinated undamaged polypeptides to be refolded to the native state assisted by ATP-fuelled disaggregating chaperones such as HSP110-HSC70 [45, 84, 85] (fig. 2 step 4).

Aggresomes are only one type of cellular deposition sites among others described as insoluble protein deposits (IPODs), juxta-nuclear quality control compartment (JUNQ) [86], also named intranuclear quality control compartment (INQ) [87] and the endoplasmic-reticulum-to-Golgi intermediate compartment (ERGIC) [88]. It should be noted that once such a late stage of protein clearance has been reached, in which toxic conformers are already effectively compacted into less toxic cellular deposits, overdue attempts to reduce protein misfolding toxicity by expressing artificially a powerful disaggregating chaperone, might produce counterproductive effects. Illustrating the possible contradictory actions of a chaperone at different concentrations on already formed compact aggregates, Shorter and Lindquist showed that above physiological levels of HSP104 in yeast could dramatically decrease the cellular levels of SUP35 prions, yet without completely curing them, whereas, HSP104-knockout mutants were fully resistant to SUP35 infection and propagation [89].

Thus, at the later stages of protein homeostasis where least toxic protein deposits are already formed, a more advantageous path would be to form an autophagosome around the intracellular protein deposits, to then be fused with the lysosome in a process called macro-autophagy (fig. 2 step 5). The peptides that are produced by the lysosome and the proteasome can be further hydrolysed by peptidases into amino acids which will be reused for protein synthesis.

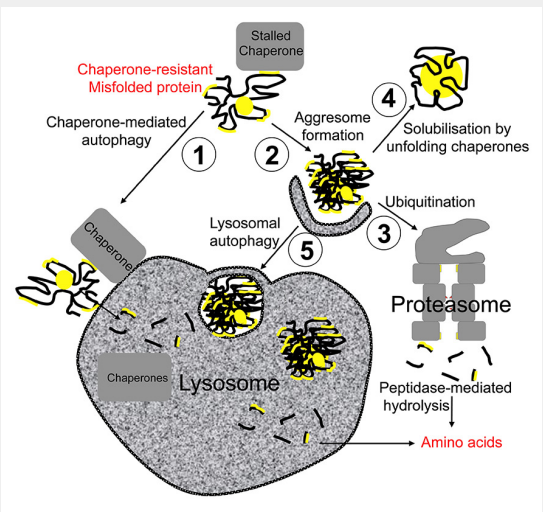

Figure 2

Compaction and degradation of irreversibly damaged polypeptides by the lysosome and the proteasome. When an irreversibly damaged polypeptide is over-sticky and is stalling an unfolding chaperone such as HSC70, the chaperone can target the polypeptide to the lysosome surface (step 1), where it will be specifically imported by specific transporters and degraded by chaperone-mediated autophagy. Alternatively, it may be compacted by the aggresome machinery (step 2), ubiquitinated, unfolded and degraded by the proteasome (step 3). When not ubiquitinated, the polypeptide may be actively disaggregated by unfolding chaperones and reach the native state (step 4). Alternatively, proteasome-resistant aggresomes may become enveloped by autophagosome membranes and imported into the lysosome to be degraded by autophagy (step 5).

Conclusion

Aging is a natural and necessary degenerative process of the somatic cells, leading to death. Young embryonic, germline and cancer cells are potentially immortal by virtue of their ability to massively produce optimal defences to protect, repair and replace their delicate macromolecules, such as DNA, proteins and lipids that may accumulate structural and chemical damages, as time passes. Depending on the tissues, the sum of masses of the molecular chaperones, co-chaperones, of chaperone-gated proteases, and of the proteins involved in micro- and macro-autophagy may reach 20% of the total proteins [47]. Furthermore, somatic cells may need to invest in cellular defences, albeit only for a defined period of time, mostly before and less beyond, reproductive age [13, 15].

Many questions remain unanswered, such as what is the nature of the signal that is apparently sent from the germline cells to repress the expression of cellular defences in somatic cells, and causing protein homeostasis collapse and death? Another question is what distinguishes young embryonic cells with optimal quality control mechanisms that are growing and differentiating into healthy pre-adult individuals from cancer cells with seemingly optimal quality control mechanisms that are growing into unhealthy tumours? Answers may lead to the development of genetic or pharmacological treatments to reactivate specific unfolding-disaggregating chaperones, co-chaperones and proteases, to retard the onset of protein-misfolding diseases and aging without causing cancers. This knowledge could also lead to the development of new anticancer drugs, HSP70 and HSP90 inhibitors in particular, that would induce the collapse of proteostasis and apoptosis specifically in rapidly dividing and growing cancer cells and not in healthy aging quiescent cells [90].

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Figures (large format)

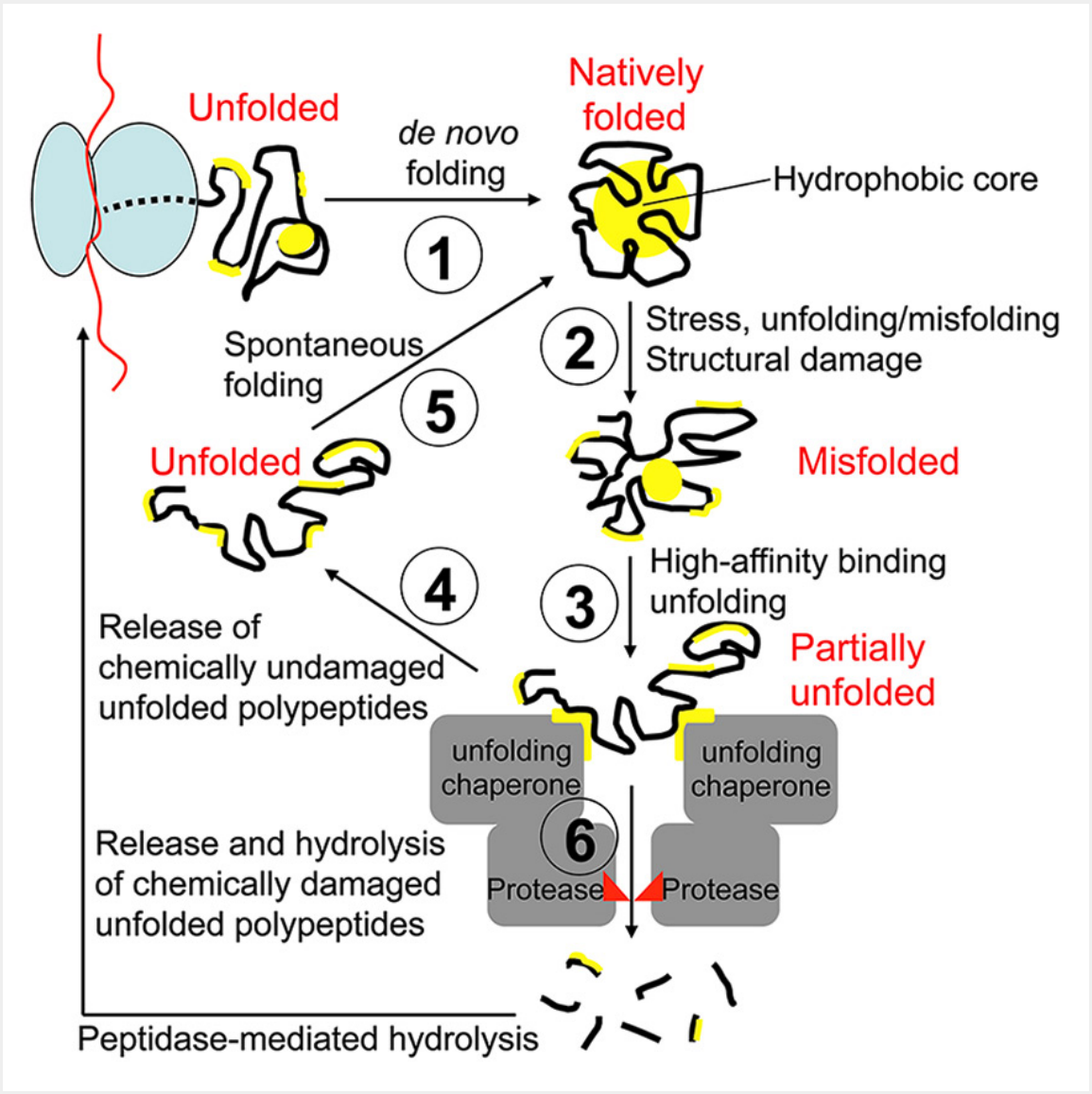

Figure 1

Proteins have an eventful life between birth on the ribosome and death by degradation. Nascent polypeptides with exposed hydrophobic residues (yellow) may spontaneously fold to the native state (step 1) with most hydrophobic residues forming a stable hydrophobic core. Stresses may cause their partial unfolding and misfolding with exposed hydrophobic residues (step 2) that can specifically bind (step 3) the hydrophobic surfaces (yellow) on unfolding chaperones (deep grey) and unfold. Following unfolding some polypeptides can be released (step 4) to spontaneously refold to the native state (step 5). Alternatively, over-sticky unfolded polypeptides can be funnelled into the proteolytic chamber (red triangles) of a protease that is associated with the chaperone (step 6) and degraded into small peptides and by peptidases into amino acids to be reused in *de novo* protein synthesis.

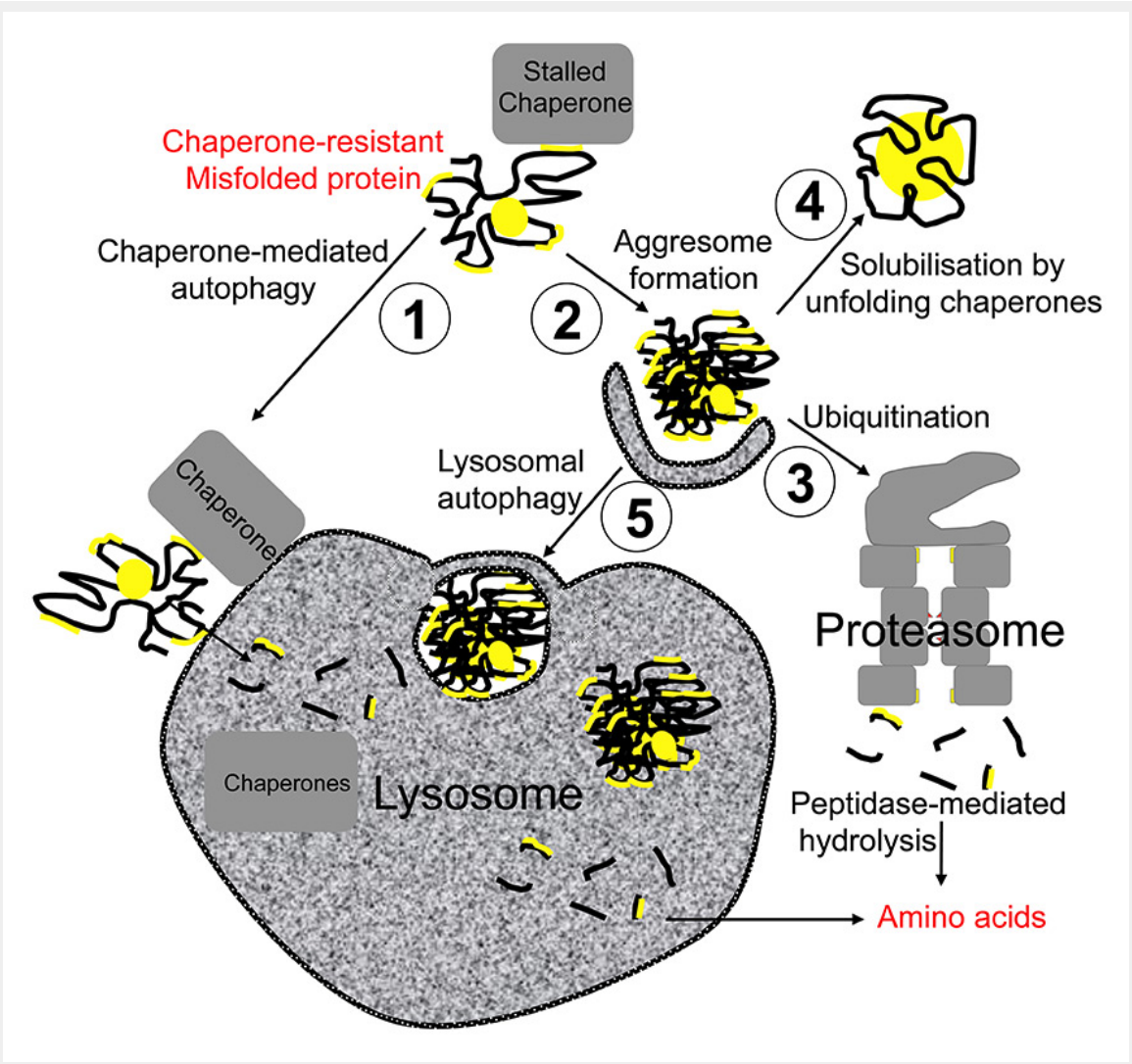

Figure 2

Compaction and degradation of irreversibly damaged polypeptides by the lysosome and the proteasome. When an irreversibly damaged polypeptide is over-sticky and is stalling an unfolding chaperone such as HSC70, the chaperone can target the polypeptide to the lysosome surface (step 1), where it will be specifically imported by specific transporters and degraded by chaperone-mediated autophagy. Alternatively, it may be compacted by the aggresome machinery (step 2), ubiquitinated, unfolded and degraded by the proteasome (step 3). When not ubiquitinated, the polypeptide may be actively disaggregated by unfolding chaperones and reach the native state (step 4). Alternatively, proteasome-resistant aggresomes may become enveloped by autophagosome membranes and imported into the lysosome to be degraded by autophagy (step 5).