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Review Article

It's not just a phase; ubiquitination in cytosolic protein quality control

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The accumulation of misfolded proteins is associated with numerous degenerative conditions, cancers and genetic diseases. These pathological imbalances in protein homeostasis (termed proteostasis), result from the improper triage and disposal of damaged and defective proteins from the cell. The ubiquitin-proteasome system is a key pathway for the molecular control of misfolded cytosolic proteins, co-opting a cascade of ubiquitin ligases to direct terminally damaged proteins to the proteasome via modification with chains of the small protein, ubiquitin. Despite the evidence for ubiquitination in this critical pathway, the precise complement of ubiquitin ligases and deubiquitinases that modulate this process remains under investigation. Whilst chaperones act as the first line of defence against protein misfolding, the ubiquitination machinery has a pivotal role in targeting terminally defunct cytosolic proteins for destruction. Recent work points to a complex assemblage of chaperones, ubiquitination machinery and subcellular quarantine as components of the cellular arsenal against proteinopathies. In this review, we examine the contribution of these pathways and cellular compartments to the maintenance of the cytosolic proteome. Here we will particularly focus on the ubiquitin code and the critical enzymes which regulate misfolded proteins in the cytosol, the molecular point of origin for many neurodegenerative and genetic diseases.

Introduction

The proper functioning of proteins in cells is critical for all forms of life. Proteins serve as the molecular machines that facilitate a wide array of life-giving processes. The normal cellular function of these molecular machines is contingent on the proper folding of newly translated polypeptides, the assembly of protein complexes and the degradation of aberrant proteins. For proteins to carry out their specialised function, they must reach a favoured native conformation [1]. To achieve this state, proteins must properly fold with the assistance of molecular chaperones and cofactors. Under normal conditions, this can already be challenging as protein biogenesis is an error-prone process [2]. Additional factors can also hinder protein folding such as mutations and environmental stresses including heat shock, metabolic stress and oxidative changes. The perturbation of cellular homeostasis has implications for degenerative conditions with mounting evidence linking the accumulation of misfolded proteins and aggregates to disease (Summarised in Table 1).

To overcome these challenges, extensive protein quality control (QC) mechanisms have evolved to oversee the fine balance between protein folding and degradation. Neutralising misfolded cytosolic proteins is a concerted effort between chaperones, the ubiquitin-proteasome system (UPS) and subcellular quarantine (Figure 1). Each of these pathways acts as a failsafe against the accumulation of toxic misfolded proteins and ablation of these critical QC pathways can result in numerous diseases. Protein QC pathways have distinct mechanisms for different subcellular circumstances including ER-associated protein degradation (ERAD), the mitochondrial unfolded protein response and ribosomal QC. Each of these pathways co-opts unique molecular machinery and E3 ubiquitin ligases to

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Table 1 Overview of key components found to be involved in cytosolic protein quality control and corresponding disease-associated mutations

Protein	Pathological condition	Genetic mutation	Molecular mechanism	References
CHIP	Cerebellar Ataxia Gordon–Holmes syndrome	Various missense mutations and truncations	Mutations in CHIP's catalytic U-box domain or TPR binding domain result in impaired function	[3–5]
UBR4	Episodic Ataxia Hirschsprung disease	R5091H missense mutation, transcriptionally down-regulated	Mutation in C-terminal end of protein associated with familial ataxia, transcriptional down-regulation in Hirschsprung disease	[6,7]
HUWE1	Say–Meyer syndrome Intellectual Disability	Splice variant Various missense mutations	Mutations in highly conserved regions of the gene, likely critical to function	[8–11]
Ataxin-3	Machado–Joseph disease	Poly-Q expansions	Poly-Q expansions result in the misfolding of Ataxin-3	[12]
Ubiquilin-1	Alzheimer's disease	Various single nucleotide polymorphisms	Ubiquilin-1 mutation associated with familial Alzheimer's disease, disputed in literature	[13,14]
Ubiquilin-2	ALS, Frontotemporal dementia	Various missense mutations	Mutations in *UBQLN2* gene cause dominant inheritance of ALS, Mutations in *UBQLN2* also impair binding to FUS which affects the ability of Ubiquilin-2 to regulate stress granule formation	[15–18]
VCP	ALS, Paget's Disease (IBMPFD)	Various missense mutations	Multiple missense mutations spanning multiple exons in conserved regions of VCP	[19–22]

maintain proteostasis and these pathways are reviewed eloquently elsewhere [23–25]. Cytosolic protein QC is of particular importance due to its links to neurodegenerative conditions including Amyotrophic Lateral Sclerosis (ALS), Huntington's, Parkinson's and Alzheimer's Diseases. Thus an understanding of cellular protein QC and the pathways that can be leveraged to combat cytosolic protein misfolding will be key to designing future therapeutics for these debilitating diseases.

Chaperones in quality control

Cells are exposed to numerous stresses that perturb the finely tuned balance of the proteome and lead to errors in protein folding and complex assembly. These stresses are varied and include changes in temperature, oxidative stress and inhibition of QC pathways, each of which elicits a different response [26,27]. These perturbations can be disruptive to proteins in the cytosol, an environment in which molecular crowding and an absence of lipid membranes means the errant display of hydrophobic patches is energetically unfavourable [28]. Molecular chaperones act as the first responders to imbalances in proteostasis, sequestering and attempting to catalyse the refolding of damaged proteins. Many of these chaperones are named Heat Shock Proteins (Hsp) after their crucial role in the heat shock response pathway [29]. The prominent chaperone Hsp70 recognises damaged proteins by their exposed hydrophobic patches [30–32], whilst another class of chaperones, Hsp90 proteins, have been shown to recognise clients for refolding by their intrinsic instability [33]. If able, the chaperones will correct the misfolding, yielding a functional protein. However, in many circumstances the problem of protein misfolding cannot be remedied by chaperone-mediated refolding alone. This can be because a protein contains an amino acid substitution that prevents it from folding appropriately or from forming appropriate protein complexes. Indeed, approximately half of all missense mutations sampled showed a defect in correct protein complex assembly [34].

Chaperones are also limited by the requirement for numerous cofactors to facilitate protein client engagement such as Bcl-2-associated Athanogene (BAG) family proteins that assist Hsp70 chaperones [35]. Under

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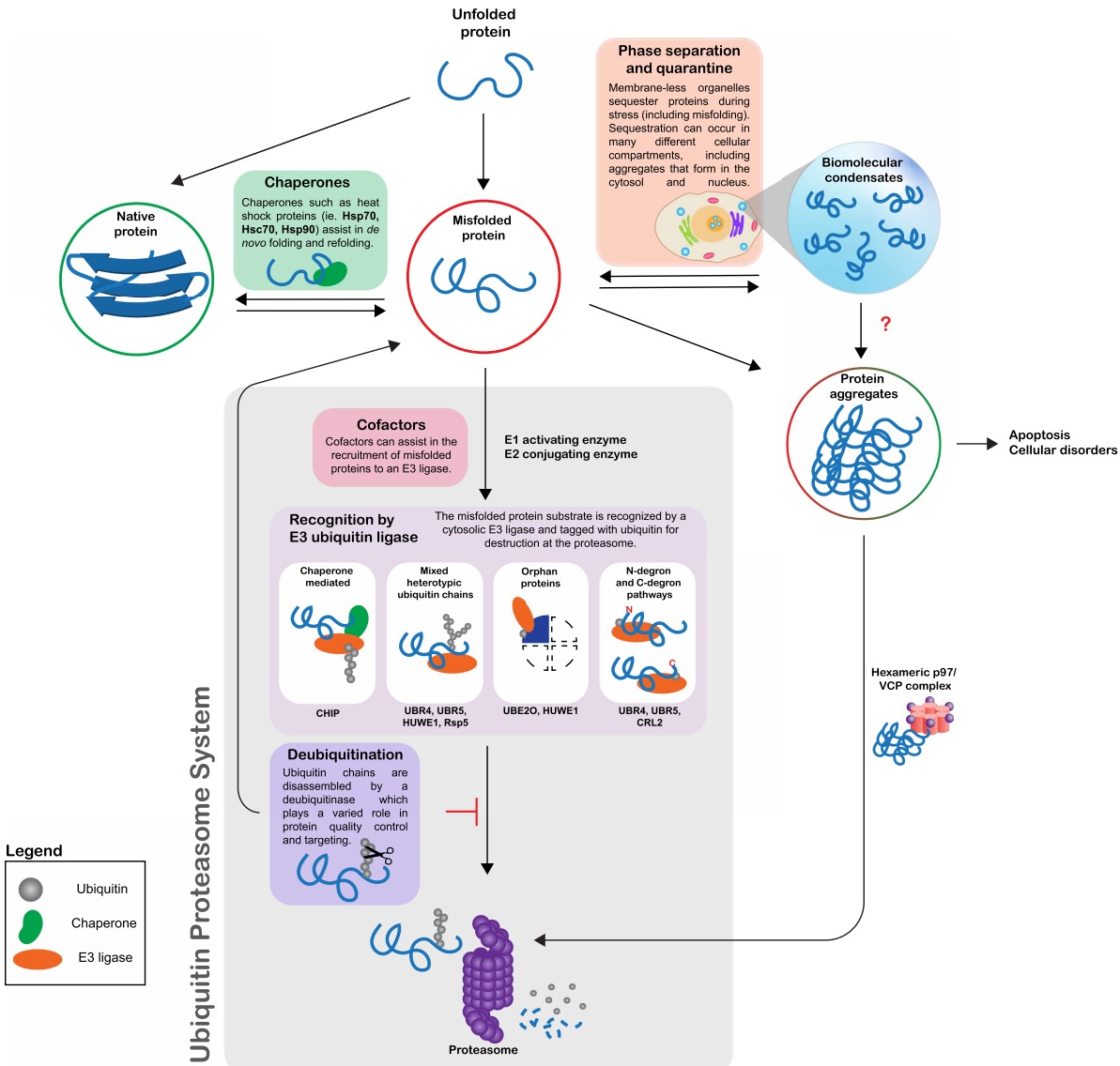

Figure 1. Schematic representation of the molecular fates and key enzymatic regulators of misfolded proteins. Chaperones (green) and ubiquitin ligases (orange) act in concert to refold or destroy misfolded proteins. In some contexts, misfolded cytosolic proteins may form inclusions either as phase separated compartments or insoluble aggregates. The cross-talk between these two states remains largely unknown.

conditions of acute stress, the limit of refolding by chaperones and cofactors is most evident, as refolding cannot always rapidly respond to the load of damaged proteins. Whilst there is evidence of chaperone networks becoming rewired in ageing and differentiation [36,37], refolding alone is insufficient to remedy all stresses. In these cases, the first-responding chaperones temper the cellular response to damaged proteins, pivoting from energy-intensive refolding to directing misfolded proteins to the UPS, autophagic machinery or other cellular compartments in both yeast and mammalian systems [38–42]. One key role that chaperones have in this context is to assist the UPS in targeted protein destruction.

Proteostasis and ubiquitin

The persistence of misfolded proteins is a significant risk to cells as well as an inefficient use of resources. These misfolded intermediates can occupy the chaperone machinery despite being terminally damaged.

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Additionally, whilst these inefficient proteins linger in the cell, their amino acids cannot be recycled for new protein synthesis. The UPS acts as an efficient system to triage these misfolded proteins by the stepwise conjugation of the small protein modifier, ubiquitin, onto target proteins. This involves an initial charging and transfer of ubiquitin to a cysteine-reactive E1 enzyme which then passes ubiquitin to a ubiquitin-conjugating E2. E2 enzymes then catalyse the transfer of ubiquitin onto substrates via E3 ubiquitin ligases. Really Interesting New Gene (RING) E3 ligases provide a molecular scaffold for substrates to be ubiquitinated by charged E2s whereas RING-between-RING (RBR) and Homologous to E6-AP (HECT) E3 ligases require the formation of a ubiquitin-thioester intermediate on the E3 prior to ubiquitination of the substrate [43,44]. The complexity of this cascade is evident in the many unique E1, E2 and E3 enzymes that execute this process with the human genome encoding more than 600 distinct E3 ligases [45].

A single ubiquitin can be deposited on substrates (termed monoubiquitination) but often E3 ligases assemble chains with additional ubiquitins conjugated in a stepwise fashion onto the lysine residues or the N-terminal methionine of the adjacent ubiquitin. Ubiquitin chains assembled on lysine 48 (K48) typically target a protein to the proteasome for destruction whereas other chain linkages, such as K63 or K11, have been described to have roles in signalling and protein complex assembly [46–48]. Further complexity exists within the ubiquitin code, with branched chains also fine-tuning ubiquitin signalling (reviewed in [49]). Heterotypic ubiquitin chains have been shown to fast-track proteins for degradation under stress with K48/K11 [50] and K48/K63 hybrids involved in the cellular response [51]. This suggests that there are potentially multiple E3 ubiquitin ligases that edit the ubiquitin code to tune the cell's cytosolic protein QC response.

Once tagged for degradation, typically by K48 chains, substrates then dock at the proteasome by making contacts with Ubiquilin family members tethered to the proteasome. The Ubiquilins bind to ubiquitin chains on substrates by their ubiquitin-binding domains and are able to then direct proteins to the proteasome for digestion. Whilst the mechanistic importance of Ubiquilin-1 has been interrogated in Alzheimer's disease model systems [52], clinical studies are less certain on the role of these proteins in the patients [13,14,53,54]. Likewise, Ubiquilin-2 mutants cause defects in protein QC in ALS, with defective processing of substrates and assembly of stress granules [15–18]. The binding of ubiquitin by Ubiquilins is key to coordinating the cellular response to misfolded proteins by recruiting QC machinery including autophagy components and proteasomes to misfolded protein condensates [55]. The interplay between ubiquitin and these distinct cellular fates in protein misfolding is an area of active research and is discussed below.

Ubiquitination and chaperones

When chaperones cannot refold their clients, they can direct them for degradation by recruiting E3 ubiquitin ligases. One such prominent E3 ligase involved in cytosolic protein QC is C-terminus of Hsc70-interacting protein (CHIP) [56]. CHIP (also known as STUB1) selectively ubiquitinates misfolded substrates via its U-box domain [57], which requires the recruitment of clients by molecular chaperones including Hsp90 and Heat Shock Cognate 70 (Hsc70) [56,58]. In combination with its chaperone partners, CHIP targets substrates for degradation via proteasomal degradation [59]. The phosphorylation of CHIP by Protein Kinase G has also recently been shown to increase the activity of the E3 ligase by enhancing its association with chaperones [60]. CHIP does not exclusively tag proteins for destruction however, it also coordinates misfolded protein aggregation in concert with its chaperone partners upon proteasome inhibition, although this may be an indirect effect [61]. In a similar vein, CHIP activity is vital to coordinating the cell's response to heat shock, suppressing apoptosis and activating Heat Shock Factor 1 (HSF1)-mediated transcription [62].

CHIP defects have been identified in multiple neurodegenerative conditions including Alzheimer's Disease as it targets phosphorylated Tau via its bound chaperone, Hsc70 [63–65]. Tau is an Alzheimer's related protein which can cause neurofibrillary tangles and its regulation by the UPS may prevent its pathological aggregation. Disease-associated mutations in CHIP further stress its importance in proteostasis with numerous reports of degenerative ataxias and neuronal decline resulting from mutations in its catalytic U-box domain or its Tetratricopeptide Repeat (TPR) binding domain that binds its chaperone partners [3–5,66]. CHIP is by no means the only mammalian ubiquitin ligase responsible for maintaining cytosolic homeostasis however, with other E3 ligases also implicated in cytosolic protein QC.

Ubr family and degron sequences

The N-recognin family of proteins are able to recognise their protein substrates of interest via a destabilising N-terminal motif (N-degron) and have a key role in regulating protein lifespan. The N-recognin family

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contains a set of proteins that bind the N-degron using a zinc-finger-like motif in their Ubiquitin-Protein Ligase E3 Component N-Recognin (UBR) boxes [67–69]. These proteins are associated with substrate ubiquitination and a number of the family members contain HECT or RING domains for this function [70]. Recent work has highlighted these proteins can degrade misfolded proteins in addition to proteins with N-degrons. The UBR family was originally characterised in cytosolic protein QC in yeast with the E3 ligase Ubr1 identified as the key degrader of model misfolded proteins in a chaperone-dependent manner [71,72] with similar observations subsequently made in mammalian cells [73]. Likewise, the E3 ligase function of family members UBR4 and UBR5 has also been co-opted by the cell for the proteasomal-degradation of misfolded proteins [50,51,74]. While UBR5 is thought to ubiquitinate substrates through its HECT domain [75], the mechanism by which UBR4 facilitates ubiquitination is currently not well understood. Additionally, UBR4 has been implicated in disease pathways related to neurodegeneration, including Pten Induced Kinase 1 (PINK1) processing [70,76]. PINK1 is a crucial enzyme for mitophagy, with defects causing Parkinson's Disease [77]. Much like CHIP, UBR4 also has a clear role in neuronal homeostasis with perturbations in the gene having detrimental effects on neurons [6,7,78–80].

UBR5 plays a unique role in the UPS by generating mixed polyubiquitin chains to target protein substrates for degradation. UBR5 has been shown to be recruited to assemble K48/K63 branched ubiquitin chains to tag substrates for degradation [50,51]. The mechanism of UBR5 is somewhat unconventional as it modifies preformed K63 chains with K48 linkages to form heterotypic branched chains. UBR5 was also found to be associated with K11/K48 branched ubiquitin chains which accelerate proteasome-dependent degradation [81]. Notably, UBR5 does not act alone as it requires basal ubiquitination by various other E3 ligases to assemble these branched chains for successful protein QC [51].

In addition to the UBR E3 ligases in the N-degron pathway, a mechanism termed DesCEND (destruction via C-end degrons) has recently been described, in which the Cullin RING Ligase 2 (CRL2) complex ubiquitinates proteins with truncations by recognising C-terminal glycine and arginine containing-motifs [82]. Like other Cullin complexes, CRL2 requires cofactors to facilitate protein degradation, including the BC-box containing adaptors which direct specific clients to the complex for degradation [82]. DesCEND complements the UBR family proteins described above, maintaining proteostasis by triaging proteins with inappropriate truncations. Whilst these molecular mechanisms counteract the incomplete folding of polypeptide chains, the cell must also cope with the incorrect assembly of proteins into higher-order complexes and this requires a further set of molecular machinery.

Protein complexes

Many proteins are required to assemble into multi-subunit protein complexes to fulfil their cellular function. The incorrect assembly of these complexes can be detrimental to the cell however as it renders the complexes dysfunctional and prone to aggregation due to non-specific interactions [83]. A quality control pathway for protein dimerisation was recently described, where aberrant protein dimers were selectively targeted by a Cullin E3 ligase complex (SCF^{FBXL17}) for proteasomal degradation [84]. Similarly, quality control pathways for subunits of unassembled protein complexes (termed orphan proteins) have also been identified. Under normal conditions, orphan proteins comprise a significant amount of proteins targeted by the protein QC system [85]. Unlike prokaryotes which utilise operons to ensure the synthesis of related proteins in a coordinated manner, eukaryotes have a much more complicated cellular physiology which can lead to unassembled proteins [86,87]. The selective targeting of orphan proteins has been shown in the assembly of haemoglobin, a tetrameric complex composed of two α-globin subunits and two β-globin subunits, where mutations in haemoglobin result in dissociation of the complex and destruction of orphans to maintain proteostasis [88–90].

The ubiquitin-conjugating (E2) enzyme (UBE2O) was identified as an integral component of the protein QC network that targets orphans of multiprotein complexes for degradation [91]. UBE2O was found to target nascent ribosomal proteins (including ribosomal proteins of large subunit [RPL] RPL3, RPL8, and RPL24) that fail nuclear import or assembly into the ribosome [91,92]. UBE2O is also implicated in the remodelling of the reticulocyte proteome upon maturation, where hundreds of different pre-existing cytosolic proteins are targeted for degradation via the UPS through multi-monoubiquitination [92]. UBE2O is required for the degradation of unassembled α-globin and is able to recognise the substrate via exposed hydrophobic surfaces. Thalassemias, a group of hereditary blood disorders, are the result of an imbalanced synthesis of haemoglobin subunits resulting from mutations and are a common set of diseases involving orphan proteins [93,94]. Although the

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reduction in haemoglobin is critical in driving the pathogenesis of these diseases, it has been suggested that orphan α- and β-globin can have toxic cellular effects, highlighting the importance of the clearance of orphan proteins [95].

The E3 ligase HECT, UBA and WWE domain containing E3 ubiquitin protein ligase 1 (HUWE1) was also found to be involved in the degradation of orphan proteins in the cytosol [96,97]. Stable isotopic labelling by amino acids in cell culture (SILAC)-based quantitative mass spectrometry identified HUWE1 substrates as both nuclear and cytosolic, however HUWE1 was found to localise to small foci in the cytosol [96]. The 450 kDa protein HUWE1 contains many potential protein interacting motifs and has been shown to bind to the chaperone Hsp27. This chaperone binding may be the molecular bridge by which HUWE1 binds to the exposed hydrophobic regions of orphan proteins. The punctate distribution of HUWE1 throughout the cytosol may also demarcate protein QC hubs in which misfolded proteins have been hypothesised to accumulate in. Mutations in HUWE1 have also been implicated in intellectual disabilities, highlighting its potential importance in proper neuronal development [8–11].

Deubiquitinases

Whilst the assembly of ubiquitin chains by E3 ligases is vital to the targeting and degradation of misfolded cytosolic proteins, the potential effect of counteracting deubiquitinase (DUB) enzymes is less clear. DUBs counter ubiquitin signalling by remodelling and removing ubiquitin from substrates. Whilst the removal of ubiquitin chains would be expected to slow misfolded protein turnover, DUB activity was shown to be necessary for the effective targeting of misfolded proteins upon heat shock [98]. The yeast DUBs Ubiquitin Protease 2 (Ubp2) and Ubp3 were shown to trim and remodel largely non-degradative K63 ubiquitin chains to allow for the accumulation of K48 chains on substrates. A similar requirement for Ubp3 has also been reported for cytosolic protein QC by maintaining the cellular pool of free ubiquitin [99]. The CHIP-associated DUB Ataxin-3 further complicates this picture with some patients presenting with poly-Q expansions that cause Ataxin-3 itself to misfold and aggregate causing Machado–Joseph disease [12]. CHIP has also been shown to degrade Ataxin-3 [59]. Conversely, Ataxin-3 has been shown to tune CHIP activity via its DUB activity [100]. At the proteasome, the DUB Ubiquitin C-terminal Hydrolase 37 (UCH37) has also been shown to have a key role in disassembling complex heterotypic chains to allow for efficient protein degradation [101]. Given the emerging role for heterotypic chains in cytosolic protein QC, the activity of UCH37 may also have an important role in effective clearance of misfolded cytosolic proteins. Indeed, there are possibly many more DUBs that temper QC outcomes in cells that have yet to be described and the recent mapping of complex ubiquitin and chaperone networks that interconnect with the nucleus [102] also raises the question of whether ubiquitin signals are involved in the subcellular quarantine of misfolded proteins.

Compartmentalisation and phase separation

The concentration of misfolded proteins in cellular puncta has been a well-observed phenomenon in both insoluble aggregates and phase-separated compartments [103]. Whilst chaperones and the UPS act in concert to minimise the burden of misfolded proteins on the molecular QC machinery, the cell further employs compartmentalisation strategies such as aggregate formation and liquid–liquid phase separation to quarantine misfolded proteins. The cytosol is a prominent location for the subcellular quarantine of misfolded proteins. Numerous QC compartments have been described in yeast and mammals including the juxta-nuclear QC compartment (JUNQ) and insoluble protein deposit (IPOD) as well as the intranuclear QC compartment (INQ), stress granules, Q-bodies, p62 bodies and aggresome-like induced structures (reviewed in [104] and [105]). These compartments are by no means static as there is evidence of protein exchange between JUNQ and INQ [106], instead they act as molecular intersections with chaperones, UPS components and the autophagy machinery all converging here to triage damaged proteins. Particularly intriguing is how the fate of proteins in these subcellular compartments is determined. The question of which molecular signals determine these fates is the subject of ongoing inquiry.

Much like in the cytosol, there are distinct compartments in the nucleus in which misfolded proteins accumulate and are associated with chaperones [107], including the nucleolus [108]. Indeed, mutants of the ALS-associated TAR DNA binding protein 43 (TDP-43) form distinct chaperone-rich anisosomes in the nucleus which are distinct from the nucleolus [109]. These bodies contain chaperones at their core and convert into insoluble aggregates when the chaperones exhaust ATP. Protein quarantine is not confined to nuclear compartments or cytosolic bodies however, with recent evidence showing that TDP-43 can be sequestered in

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mitochondria to prevent its toxic effects [110] and misfolded Superoxide Dismutase 1 (SOD1) can dock at mitochondria also [111]. Undoubtedly, many more creative cellular solutions exist to prevent misfolded proteins from perturbing proteostasis, one of which is the formation of phase-separated QC compartments.

Liquid–liquid phase separation occurs when biomolecules concentrate due to numerous low-affinity, multivalent interactions that generate specialised loci. These loci can act as bioreactors, often having high local concentrations of enzymes that are critical to their function, such as chaperones. These membraneless phase-separated compartments are able to form in a range of contexts including in the DNA damage response, cell signalling and ribosome biogenesis [112], with the nucleolus being one such hub [113]. Whilst the nucleolus has been recently described as a QC hub during stress [108] other nuclear phase-separated sub-compartments have also been identified including nuclear speckles and Cajal bodies amongst others [114]. Strikingly, ubiquitin chains have been described as features of phase-separated compartments, including during stress [115]. Conversely, binding of ubiquitin chains can disrupt liquid–liquid phase separated Ubiquilin 2 [116]. The autophagy receptor Sequestome 1 has also been shown to colocalise with ubiquitinated proteins in phase-separated compartments [117,118], providing an elegant molecular bridge to induce autophagic clearance (reviewed in [119]). Indeed, phase separation can determine the site of autophagosome formation [120], perhaps priming phase-separated compartments for autophagy [121].

Notably both cytosolic and nuclear phase-separated compartments are fluid with the ready exchange of damaged proteins. This correlates with the predictive scoring of stress granule-associated proteins that suggests that various protein classes are capable of phase separation in different cellular locations [122]. Further work shows a reconfiguration of the localisation of QC machinery upon heat stress with chaperones accumulating in nucleoli [123]. These observations are intriguing as the proteostasis circuits that govern cytosolic and nuclear QC require distinct signals in the UPS [102]. Although this disparity requires further interrogation, it is tantalising to speculate that the ubiquitin code can divert misfolded proteins from the proteasome to the nucleolus and indeed that additional players that edit the ubiquitin code may have a role here.

Phase separation is not the only means by which misfolded proteins are quarantined, with insoluble cytosolic aggregates having been observed in numerous cell-based models and in patient samples. The UPS seemingly slows the formation of aggregates with inhibition of the proteasome leading to IPOD or JUNQ aggregate formation (reviewed in [105]). Whilst these insoluble protein deposits may accumulate, this aggregation has long been debated to be neurotoxic or neuroprotective. For example Huntingtin aggregation has been shown to arrest cell death [124] or promote apoptosis [125].

It is important to note, that like many of the QC pathways described here, phase-separated compartments and protein aggregates are constantly being tuned by cells and are not fixed entities. The ubiquitin-dependent segregase Valosin-containing protein (VCP) (also known as p97) is a salient example of this as it has a prominent role in disassembling protein aggregates as well as tuning ribosomal QC [126] and modulating ERAD through cytosolic phase-separated microcompartments [127]. Indeed, VCP is critical for extracting ubiquitinated proteins from aggregates [128] and facilitating their degradation via the proteasome. There are also numerous binders of the hexameric VCP oligomer with varied cellular roles, pointing to an interconnectedness and concerted response between VCP and DUBs, the UPS and autophagy [129,130]. VCP acts as a central node, coordinating the cellular response to protein aggregation. This is underscored by the pathophysiology of inclusion body myopathy, Paget disease of the bone and frontotemporal dementia (IBMPFD) in patients with mutated VCP [19–22,131]. Variants of VCP cause the accumulation of ubiquitinated proteins and mislocalise misfolded proteins [131]. Further work will be required to untangle whether aggregation is a by-product of overwhelmed protein QC mechanisms or an alternate defence strategy for shielding cells from toxic misfolded proteins in the cytosol. The example of VCP however, suggests that aggregates may only be intermediate proteinaceous deposits that are awaiting extraction by VCP and subsequent degradation.

Future directions

The complexity of protein QC outlined in this review raises important questions about how distinct cellular fates are induced. The mechanism of whether a misfolded cytosolic protein is degraded by the proteasome, sequestered in a subcellular compartment or targeted by autophagy is yet to be established. Indeed, multiple pathways are likely acting in concert such as when proteasomes are recruited to phase-separated compartments during cellular stress [115] or when autophagy acts in concert with protein aggregation [132]. Ubiquitin may be one such key determinant of these decisions, with evidence of ubiquitin in aggregates, phase-separated compartments and nuclear trafficking in addition to its well-understood role in the UPS. The ubiquitin-associated

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Ubiquilins are also present in proteinaceous deposits in patients with ALS and other neurodegenerative conditions [133]. Tellingly, these Ubiquilin deposits are both cytosolic and nuclear, suggesting the indispensable nature of ubiquitin signalling in protein QC throughout the cell [134,135]. It is likely that these distinct cell fates are operating synchronously with particular pathways induced or suppressed according to the cellular context. Chaperones, in addition to ubiquitin, are emerging as directors of cell fate with roles in delivering cargo to the UPS, autophagy machinery or subcellular compartments. Further work will centre on the molecular signals that tune the cellular response and the thresholds required for fate determination.

Further mechanistic work is also required to delineate the intriguing observation that many DUBs and E3 ligases are mutated in degenerative conditions [136,137]. This underscores the critical requirement for a functioning UPS in healthy ageing. The association between ubiquitin and protein aggregates is of particular interest and further work will be required to delineate whether these aggregates are neuroprotective or byproducts of failed protein QC. The ubiquitin system is a promising pathway for the design of new therapies with numerous molecular pathways to target to intervene in disease. DUBs in particular have been shown to be specifically targeted by small molecules to tune ubiquitin signalling [138]. By understanding these pathways, therapeutic interventions can be tailored to skew the cellular response to rescue or cull misfolded proteins and as a consequence extend the functioning lifespan of cells that would otherwise be overwhelmed by the burden of cytosolic protein misfolding.

Perspectives

- Dysregulated protein quality control causes numerous degenerative diseases and effective therapeutics are limited for these conditions, highlighting the importance of understanding these fundamental pathways.
- The chaperone network, ubiquitin-proteasome system and cellular inclusions are the established mechanisms by which the cell controls misfolded proteins. Liquid–liquid phase separation is emerging as an additional mechanism by which cells quarantine misfolded proteins.
- Numerous pathways in protein quality control make attractive therapeutic targets with the ubiquitin-proteasome system being a prime candidate for intervention.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ALS, Amyotrophic Lateral Sclerosis; BAG, Bcl-2-associated Athanogene; BMPFD inclusion body myopathy, Paget disease of the bone and frontotemporal dementia; CHIP, C-terminus of Hsc70-interacting protein; CRL2, Cullin RING ligase 2; DesCEND, destruction via C-end degrons; DUB, deubiquitinase; ERAD, ER-associated protein degradation; HECT, Homologous to E6-AP; Hsc, Heat Shock Cognate; HSF1, Heat Shock Factor 1; Hsp, Heat Shock Proteins; IPOD, Insoluble Protein deposits; iPSC, Induced Pluripotent Stem Cell; JUNQ, Juxtanuclear Quality Compartments; PINK1, Pten Induced Kinase 1; QC, quality control; RBR, RING-between-RING; RING, Really Interesting New Gene; RPL, ribosomal proteins of large subunit; SILAC, stable isotopic labelling by amino acids in cell culture; SOD1, Superoxide Dismutase 1; TDP-43, TAR DNA binding protein 43; TPR, Tetratricopeptide Repeat; UBE2O, ubiquitin-conjugating (E2) enzyme; Ubp, Ubiquitin Protease; UBR, Ubiquitin-Protein Ligase E3 Component N-Recognin; UCH37, Ubiquitin C-terminal Hydrolase 37; UPS, ubiquitin-proteasome system; VCP, Valosin-containing protein.

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