Mechanisms and Functions of Spatial Protein Quality Control

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Abstract

A healthy proteome is essential for cell survival. Protein misfolding is linked to a rapidly expanding list of human diseases, ranging from neurodegenerative diseases to aging and cancer. Many of these diseases are characterized by the accumulation of misfolded proteins in intra- and extracellular inclusions, such as amyloid plaques. The clear link between protein misfolding and disease highlights the need to better understand the elaborate machinery that manages proteome homeostasis, or proteostasis, in the cell. Proteostasis depends on a network of molecular chaperones and clearance pathways involved in the recognition, refolding, and/or clearance of aberrant proteins. Recent studies reveal that an integral part of the cellular management of misfolded proteins is their spatial sequestration into several defined compartments. Here, we review the properties, function, and formation of these compartments. Spatial sequestration plays a central role in protein quality control and cellular fitness and represents a critical link to the pathogenesis of protein aggregation-linked diseases.

Contents

INTRODUCTION

The ability of the cell to maintain functional proteins is critical to cell survival (1, 2). Cells have evolved complex protein quality control (PQC) systems comprised of multiple chaperone and clearance pathways that cooperate to preserve protein homeostasis (3–6). Disruption of effective PQC is linked to many human diseases, including neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis (ALS) but also metabolic diseases, lysosomal storage diseases, cancer, and aging. Because aggregates of proteins misfolded to the β-sheet-rich amyloid state are hallmarks of many neurodegenerative disorders (7–10), there has been keen interest in understanding the cellular and structural bases of protein aggregation. The recent evidence that cells actively promote sequestration of misfolded protein into inclusions is challenging the simple view that the aggregates themselves are pathogenic.

Protein folding and assembly begins during translation, often with the assistance of cotranslationally acting chaperones to achieve the native conformation (6, 11). However, newly made proteins can fail to fold, for instance, if they lack a binding partner, contain mutations or truncations, or fail to reach the proper cellular compartment (3). Proteins also misfold due to exposure to damaging environmental conditions such as elevated temperature, pH, or reactive oxygen species (6, 12–14). Misfolded proteins can have a number of deleterious effects in cells due to loss of function of a required protein or toxic gain-of-function effects of the misfolded conformation (3, 15).

PQC: protein quality control

Once a protein is misfolded, it can be refolded, degraded, or terminally sequestered. The PQC system consists of several types of molecular chaperones and degradation systems that work together to ensure a healthy and functional proteome (13, 16–18). Sequestration of misfolded and aggregated proteins into distinct compartments in different locations of the cell (19, 20) was originally thought to be a secondary effect caused by failure of primary mechanisms of quality control; however, recent evidence indicates that this is an early feature of PQC in normal cells (19–21). Substantial progress has been made on the nature of spatial PQC in both yeast and mammalian cells since we first described the presence of two major sites for misfolded protein sequestration nearly a decade ago (22). In this review, we summarize the current state of this field and highlight the key areas of uncertainty that will form the focus of research for the next decade.

UPS: ubiquitin–proteasome system

IPOD: insoluble protein deposit

SPATIAL SEQUESTRATION IN DIFFERENT SUBCELLULAR COMPARTMENTS

It had long been recognized that misfolded proteins can accumulate in intracellular, insoluble inclusions. It is now clear that cells use molecular chaperones for recognition and sorting of misfolded proteins to spatially restricted subcellular compartments (19, 22–24). An increasing number of spatially distinct PQC compartments have been uncovered, and the exact function of each is still being identified (**Figure 1**). Some PQC compartments function to enhance clearance by the ubiquitin–proteasome system (UPS) or autophagy, whereas others appear to terminally sequester aggregation-prone insoluble proteins (22, 25, 26). From a conceptual perspective, spatial sequestration fulfills two additional important functions, namely, to concentrate potentially toxic conformers away from the cellular milieu (21, 22) and to promote asymmetric inheritance of damaged proteins upon division so that daughter cells have a fully functional proteome (26–28). These functions appear to be essential for long-term cellular health and fitness (29–31).

Although most studies of PQC compartments have been carried out in yeast, a clear conservation across eukaryotic cells has been observed, utilizing analogous pathways and sorting factors (22, 23, 26, 32–35).

Two Major PQC Compartments for Misfolded Cytosolic Proteins

Misfolded cytosolic proteins have been shown to become sequestered in two distinct cytoplasmic PQC compartments. Sorting is dependent on the properties of the misfolded proteins, their chaperone interactions, and possibly also their ubiquitination state. One of the first compartments discovered contained insoluble aggregates, such as β-sheet-rich amyloid proteins, and was thus termed the insoluble protein deposit (IPOD) (22). The IPOD is thought to terminally sequester toxic amyloid and prion proteins in the periphery of yeast cells near the vacuole (22, 36) (**Figure 1*a***). The connection to amyloid proteins ties the IPOD to late-onset neurodegenerative diseases characterized by insoluble intracellular inclusions. Indeed, the IPOD, defined in yeast, is likely equivalent to the perinuclear aggresome described in mammalian cells as a site of sequestration for amyloid Huntingtin (Htt) (22, 33, 37). Unlike the IPOD, however, aggresomes are located at the centrosome and are surrounded by a vimentin cage.

Misfolded and stress-damaged proteins are shuttled to dynamic compartments called Q-bodies that are anchored to the endoplasmic reticulum (ER) en route to clearance by the UPS (21). Q-bodies form immediately upon misfolding, suggesting that they represent an early event in cellular PQC. Their formation is energy and chaperone dependent but cytoskeleton independent (21, 38, 39). The misfolded proteins in Q-bodies are rapidly cleared through the ubiquitin–proteasome pathway, but if clearance is impaired, these misfolded proteins concentrate in the juxtanuclear

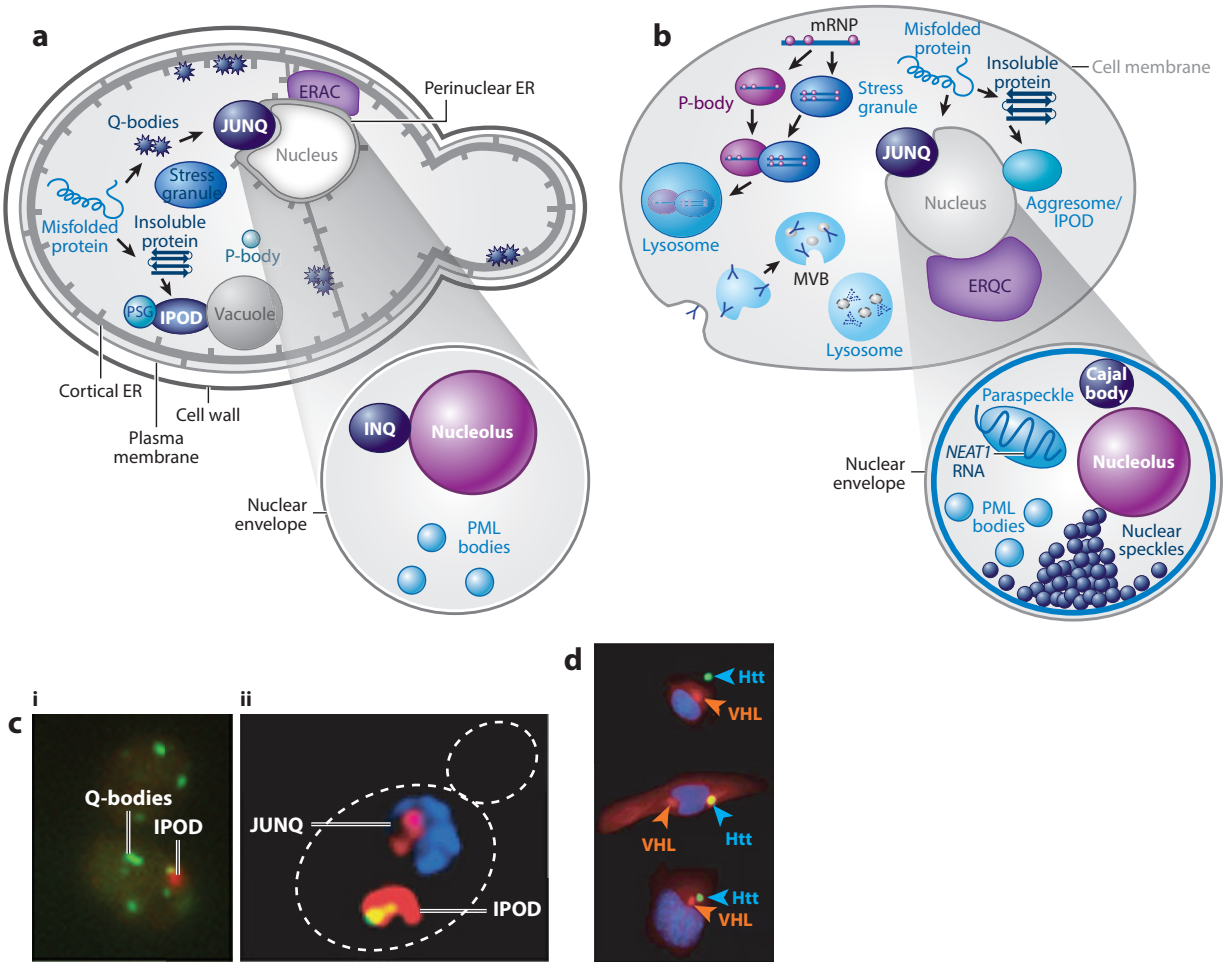

Figure 1

Protein quality control (PQC) compartments in eukaryotic cells. (*a*) Model illustrating protein misfolding and sequestration in yeast. Proteins that fold into insoluble, or amyloid, conformations are sequestered to the insoluble protein deposit (IPOD). Soluble misfolded proteins are stored in Q-bodies en route to the juxtanuclear quality control compartment (JUNQ). Other PQC compartments in the yeast cytoplasm include endoplasmic reticulum (ER)–associated compartments (ERACs), stress granules, P-bodies, and proteasome storage granules (PSGs). Proteins are sequestered into the nucleolus, intranuclear quality control compartment (INQ), and promyelocytic leukemia (PML) bodies in yeast nuclei. (*b*) Model illustrating PQC compartments in mammalian cells. Proteins are sequestered into the JUNQ, aggresome/IPOD, ER quality control (ERQC; mammalian equivalent of the yeast ERAC) system, and multivesicular bodies (MVBs). Messenger ribonucleoprotein (mRNP) complexes can be sequestered into P-bodies and stress granules en route to the lysosome for degradation. Nuclear sequestration can occur in the nucleolus, PML bodies, nuclear speckles, paraspeckles, and Cajal bodies in mammalian cells. (*c*) PQC compartments in yeast. (*i*) In the absence of proteasome inhibition, Ubc9ts–GFP (*green*) localizes to Q-bodies. Q-bodies are distinct from the IPOD [marked by Htt-Q97–CHFP (*red*)]. (*ii*) Upon proteasome inhibition, Ubc9ts–CHFP (*red*) localizes to the perinuclear JUNQ as well as to the IPOD, marked by Htt-Q103–GFP (*green*). (*d*) Quality control compartments in mammalian cells. Upon proteasome inhibition, CHFP–VHL (*red*) localizes to the perinuclear JUNQ, whereas the aggresome/IPOD is marked by Htt-Q103–GFP (*green*). Panel *c*, subpanel *i*, is adapted from Reference 21 and panel *c*, subpanel *ii*, and panel *d* are adapted from Reference 22.

quality control compartment (JUNQ) (21, 22). The JUNQ forms near the nucleus during times of stress when the proteasome is impaired (22). Proteins can diffuse in and out of the JUNQ, so this deposition site is more dynamic than the IPOD and may act as a reservoir for the storage of misfolded proteins for subsequent refolding or clearance. Of note, the JUNQ has also been observed in mammalian cells as a cytoplasmic perinuclear inclusion distinct from that formed by amyloid proteins (22, 26, 34, 40). Under conditions of severe stress or blocked ubiquitination, misfolded nonamyloidogenic proteins, such as those created by stress damage or missense mutations, can be routed to the IPOD (22) or other peripheral aggregates in an Hsp42-dependent manner (32).

JUNQ: juxtanuclear quality control compartment

Stress Granules Are Storage Sites for the Protein Translation Machinery

During stress, the cytoplasm also sequesters mRNAs and proteins involved in the translation machinery into at least two different types of compartments: P-bodies, which contain mRNA decay machinery, and stress granules, which contain several translation-initiation components (41, 42). Stress granules and P-bodies can interact and exchange messenger ribonucleoprotein (mRNP) complexes and both can serve as transient mRNP storage vesicles for later use or degradation (43, 44). Stress granules also seem to play a role in the decision to enter apoptosis during times of extreme stress (45). Although these RNA quality control compartments do not specifically sequester misfolded proteins, their formation and dissolution appear to involve chaperone regulation (42, 46–49).

It is currently unclear to what extent the cytosolic PQC compartments described here—most specifically, Q-bodies, the JUNQ, and the aggresome/IPOD—overlap with the RNA quality control compartments, P-bodies, and stress granules in terms of location, protein composition, and cellular function. However, molecular chaperones characteristically associated with Q-bodies and the JUNQ also colocalize with stress granule proteins (46, 49–51), suggesting that their formation may be coordinated during stress. Thus, multiple types of inclusions exist under physiological conditions and must form through separate pathways for sorting misfolded proteins and translation factors. Stress appears to require the deposition of these components into distinct single sites until the stress is relieved. It will be interesting to better understand the determinants for recognition and sorting of misfolded proteins, mRNA, and translation factors to these distinct subcellular locations. Mechanistically, sequestration may involve phase separation through sorting components with distinct specificities (52–57).

Sites of Nuclear PQC

Misfolded proteins can also accumulate in the nucleus (24, 58–60). The nucleus contains a number of different compartments, but the most pronounced is the nucleolus. Although traditionally seen as the site of ribosomal RNA production and ribosome biogenesis, the nucleolus is also involved in the stress response and aging (61, 62). The nucleolus becomes destabilized during stress, leading to the sequestration of the E3 ubiquitin ligase Mdm2, which can no longer bind to its target protein p53. This promotes p53 activation and expression of target genes, leading to cell cycle arrest and/or apoptosis (reviewed in 61). Fragmented nucleoli are also linked to accelerated aging in yeast with a possible link to premature aging in humans, but this appears to have more to do with genome integrity than p53 activation (63). Boulon et al. (64) provide an excellent overview of the effects of different types of stress on composition and fragmentation of the mammalian nucleolus (and Cajal bodies). Promyelocytic leukemia (PML) nuclear bodies are also punctate proteinaceous structures found in the nuclear matrix near the nucleolus (65). There are many hypotheses for the function of PML nuclear bodies, including protein storage or involvement in transcription (61, 65).

ERAD: endoplasmic reticulum–associated degradation

There are different subnuclear structures that are primarily associated with mammalian cells including nuclear speckles, paraspeckles, and Cajal bodies. Nuclear speckles are storage compartments for precursor (pre)-mRNA splicing factors and reside in the interchromatin regions of the nucleoplasm (66). Paraspeckles are ribonucleoprotein structures also found in the interchromatin region (67). They contain the long, noncoding RNA *NEAT1* as well as several proteins involved in transcription or RNA processing. They are proposed to play a role in coordinating gene expression in different cellular settings and are also involved in the regulation and posttranslational modification of p53 in cancer cells (68). Cajal bodies are made up of proteins and RNA and tethered to the nucleolus (61, 69). They are sites of assembly or modification of the transcription machinery that is involved in pre-mRNA splicing, maturation of small nuclear ribonucleoproteins (snRNPs), and pre-rRNA processing. Of particular interest, SMN is a Cajal body component critical for assembly of snRNPs in the cytoplasm and their import to the nucleus. Depletion of SMN causes spinal muscular atrophy, a neuromuscular degenerative disorder affecting the spinal cord and skeletal muscles (69). Cajal bodies have also been shown to regulate telomere length, a hallmark of aging in cells (67).

Compared to mammalian nuclei, less is known about nuclear PQC in yeast. A recent study suggested that cytosolic misfolded proteins can also be routed to an intranuclear quality control compartment (INQ) (60). The INQ is located just inside the nuclear membrane and next to the nucleolus and may aid in clearance of misfolded proteins from the nucleus (60). It is currently unclear how the INQ differs from other nuclear bodies described in mammalian cells; more work will be required to determine the distinctions between these compartments.

PQC of Membrane Proteins

There are a number of different structures associated with the ER that sequester misfolded proteins, but one of the most highly studied is the yeast ER-associated compartment (ERAC) (70), which corresponds to the ER quality control (ERQC) system in mammalian cells (71). Both the ERQC system and ERACs are networks of soluble tubule-vesicular structures that connect directly to the ER and serve as the site of sequestration for misfolded membrane proteins before clearance by ER-associated degradation (ERAD) (72).

Aside from resident ER proteins, most misfolded intrinsic membrane proteins appear to be sorted through the multivesicular body (MVB) pathway (reviewed in 73). This pathway was previously implicated in recycling activated receptors at the plasma membrane. However, it is becoming clear that misfolded plasma membrane proteins (74, 75), as well as those of the Golgi body (76), are also routed to the MVB pathway for lysosomal degradation. It should be noted here that a separate pathway, more closely related to ERAD, has been identified for misfolded proteins of the inner nuclear membrane (77).

SUBSTRATES OF SPATIAL PQC

Quality Control on the Ribosome

The last two years have witnessed great progress in our understanding of how the cell clears nascent polypeptides from defective or stalled translating ribosomes, which was reviewed recently by Brandman & Hegde (78). Elaborate mechanisms have been identified that sense problems with the translating mRNA; for example, when ribosomes stall on a defective mRNA, specialized machineries dissociate the ribosomes, target the mRNA for degradation, and recruit ubiquitin ligases that target the nascent chain of the failed ribosomes for degradation in a process now called

ribosome quality control (RQC). In contrast, still little is known about cotranslational quality control associated with evaluating the folded state of the nascent chain (79) and detecting nascent chains that fail to reach their native conformation as they emerge from the ribosome.

For RQC, the first step involves recognition of a defective or stalled ribosome. The exact mechanisms and factors involved in recognition vary depending on the type of stall, but eventually all result in ribosomal dissociation into the small subunit; the mRNA (which is degraded); and a ternary complex composed of the large subunit, the tRNA at the P-site, and the nascent polypeptide. This ternary complex is recognized by the E3 ubiquitin ligase Ltn1/Listerin (an interaction facilitated by Rqc2/NEMF), which ubiquitinates the nascent polypeptide. An additional component, Rqc2, recruits tRNAs to the dissociated 60S–nascent chain complex and attaches an alanine-threonine repeat stretch to the C terminus (known as a C-terminal alanine-threonine tail, or CAT-tail) of the nascent polypeptide. Finally, the ubiquitinated nascent polypeptide must be extracted from the 60S complex through the action of the AAA+ ATPase Cdc48 and Rqc1, before it is degraded by the proteasome.

Compromising nascent polypeptide ubiquitination (e.g., by deletion of Ltn1 or Rqc1 in yeast) triggers a stress response via activation of Hsf1 and leads to the accumulation of the CAT-tail-containing nascent polypeptides in multiple cytoplasmic puncta (80–83). By sequestering molecular chaperones and other key PQC factors, it is proposed that these CAT-tail-driven puncta are the main cause of proteotoxicity observed in ribosome-stalled polypeptides (80). Interestingly, Rqc2 appears critical for this process as inactivation of Rqc2 abrogates both Hsf1 activation and the formation of multiple cytoplasmic puncta (80–82). The effect this has on proteotoxicity in this context is unclear; however, the fact that double deletion mutants of Rqc2 and either Ltn1 or Rqc1 display no marked differences in viability from single Ltn1 or Rqc1 deletion mutants alone (80) would suggest that determinants of toxicity are more complex than puncta formation or Hsf1 activation alone.

Mistargeting of Newly Translated Proteins

Nascent polypeptides must be targeted to their correct compartment to fold. One of the best-studied paradigms for cellular misfolding is the mislocalization of tail-anchored ER membrane proteins in the cytosol. In general, membrane proteins are prone to aggregation in the aqueous environment of the cytosol due to their hydrophobic transmembrane domains. As opposed to proteins with transmembrane regions at the N terminus or within the body of the sequence—which are protected from cytosolic exposure by the signal recognition particle as the nascent polypeptide emerges from the ribosome—tail-anchored proteins are recognized posttranslationally by components of the Get/TRC pathway and subsequently inserted into the target membrane (84). Inefficient targeting leads to the accumulation of tail-anchored proteins in the cytoplasm and results in neurotoxicity such as that observed in prionopathies (85). When recognition fails, the cytosolic BAG6 complex instead binds the substrate's exposed transmembrane region and targets it for degradation via the UPS (86). However, the tetratricopeptide repeat–containing protein SGTA can antagonize BAG6-mediated ubiquitination, both by preventing BAG6 binding to the substrate and by actively triggering substrate deubiquitination (87). Therefore, this quality control system relies on a balance between prodegradative BAG6 and protargeting SGTA to determine the fate of the mislocalized membrane proteins. Consistent with this hypothesis, overexpression of SGTA delays clearance of mislocalized membrane proteins and causes them to accumulate in insoluble cytoplasmic puncta (88).

Another PQC system that is triggered by mislocalized proteins involves specific detection of mitochondrial proteins in the cytosol. In a mammalian cell, roughly 1,000 different proteins need

to be imported into mitochondria following translation (89). Inefficient mitochondrial protein import, such as that observed during aging or neuromuscular degeneration, results in their toxic accumulation in the cytosol, eventually triggering cell death (90). Recent work shows that cells can delay this toxic accumulation by simultaneously reducing protein translation and increasing proteasome activity (91, 92), similar to the unfolded protein response (UPR) induced upon misfolded protein accumulation in the ER lumen (93). In the case of mitochondrial membrane proteins, it has recently been proposed that ubiquilins play a role in both targeting and triage (94). The mechanism of luminal mislocalized mitochondrial protein recognition remains uncharacterized; it does not seem to induce transcriptional responses associated with the general accumulation of misfolded proteins in the cytosol, such as the heat shock response (91). Therefore, this pathway likely represents a specific PQC system that buffers against cell death when mitochondrial dysfunction is transient.

Stress-Induced Misfolding

In yeast, transcriptome analysis reveals that 900 genes (~10–15% of the genome) are similarly modulated by a wide range of environmental stresses (95). In the broadest terms, the common theme among these core stress-modulated genes is the downregulation of protein synthesis and cotranslationally acting chaperones and the upregulation of stress-inducible chaperones and degradation components, which presumably protects the proteome from stress (95, 96).

Protein structure is generally fragile, and a variety of environmental stresses, including oxidative stress and heat, can damage or destabilize labile protein structures. Heat shock leads to a clear accumulation of protein aggregates (97). Newly translated proteins—which haven't yet reached their native conformation—are rapidly degraded upon heat shock, with the bulk of folded proteins being comparatively less affected (98). However, mutations that destabilize protein structure will render proteins that are at the boundary of stability highly vulnerable to misfolding during stress, leading to loss of function and aggregation (22, 99, 100, 101). The trade-off among stability, aggregation propensity, and abundance likely imposes an evolutionary drive to reduce the concentration of aggregation-prone proteins (102, 103); however, this delicate balance can be disrupted by stress.

Heat shock also promotes the sequestration of proteins that remain folded. A recent proteomic study identified around 170 endogenous proteins that moved from the soluble to insoluble cellular fraction upon heat shock (51). None of the proteins in the insoluble fraction were misfolded, and all were recovered in the soluble fraction upon relief from heat shock. Although the authors concluded that heat shock–induced aggregates are fully reversible and not in fact turned over by the UPS or autophagy as was previously hypothesized, it is also possible that the mild sedimentation conditions used here—which differed strongly from approaches normally used to identify bona fide aggregates (104–106)—led to identification of proteins that upon heat shock move to sedimentable inclusions, such as stress granules, which are much more abundant than heat-labile proteins that aggregate. Indeed, there is an emerging understanding that stress, stationary phase, or starvation, in addition to destabilizing labile proteins, induces phase-separation events that store folded metabolic enzymes and other cellular complexes as a protective mechanism (54, 107, 108).

MECHANISMS AND DETERMINANTS OF SPATIAL PQC

The existence of several distinct spatial PQC pathways and compartments acting on distinct types of misfolded proteins raises the question of the identity of the factors and determinants involved in these decisions. Recognition of misfolded proteins may involve a common set of chaperones, such as Hsp70s, but the location and nature of the misfolded protein may also recruit a specialized

Table 1 Molecular chaperones implicated in spatial protein quality control

Chaperone	Family	Compartment	Role in protein quality control	Reference(s)
Ssa1/2 *Hsc70/Hsp72*	Hsp70	Q-bodies; JUNQ/INQ; stress granules	Refolds proteins in Q-bodies; Q-body coalescence; with Sis1 sorts to INQ; stress granule clearance	21, 22, 24, 49
Hsc82/Hsp82 *Hsp90α/β*	Hsp90	Q-bodies	Refolds proteins in Q-bodies; Q-body coalescence	21
Sse1	Hsp110	Q-bodies	Refolds proteins in Q-bodies; Q-body coalescence; Q-body clearance; substrate ubiquitination	21, 132
Sti1 *Hop*	Sti1	JUNQ/INQ	Sorting factor; JUNQ targeting and clearance	22, 60
Ydj1	Hsp40	Q-bodies; stress granules	ER-associated sorting factor; Q-body formation; stress granule disassembly; Rsp5-mediated degradation upon heat shock	21, 49, 158
Sis1	Hsp40	JUNQ/INQ; IPOD; stress granules	Sorting factor; with Btn2 sorts to JUNQ; with Cur1 sorts to INQ; with Hsp104 sorts to IPOD; with Hsp70 sorts to INQ; proteasomal degradation of cytosolic substrates; vacuolar targeting of stress granules	23, 24, 49, 60, 114
Hsp26	sHSPs	JUNQ/INQ; IPOD	Sorting factor under severe heat shock; disaggregation of heat-denatured proteins	32, 124
Hsp42	sHSPs	Q-bodies; IPOD	Sorting factor; with Btn2 sorts to IPOD/periphery	21, 23, 32
Hsp104	Hsp100 (AAA+ ATPase)	Q-bodies; JUNQ/INQ; IPOD	Disaggregase; with Sis1 sorts to IPOD; JUNQ clearance after removal of stress	21–23, 32, 60
Btn2	Hook	JUNQ/INQ; IPOD	Sorting factor; with Hsp42 sorts to IPOD; with Sis1 sorts to JUNQ	22, 23, 32, 60
Cur1	Hook	INQ	Sorting factor; with Sis1 targets to INQ	23
Cdc48 *p97/VCP*	AAA+ ATPase	INQ; ERAD; stress granules	"Extractase"/disaggregase; extracts ubiquitinated membrane proteins for ERAD; keeps aggregation-prone nuclear proteins soluble for ubiquitination; autophagic clearance of stress granules	47, 146

Abbreviations: ER, endoplasmic reticulum; ERAD, ER-associated degradation; INQ, intranuclear quality control compartment; IPOD, insoluble protein deposit; JUNQ, juxtanuclear quality control compartment; sHSP, small heat shock protein.

machinery for sorting to a given PQC compartment. In this section, we describe what is currently known about how a cell sorts misfolded proteins into distinct PQC compartments.

Molecular Chaperones Are Key Regulators of Spatial PQC

Molecular chaperones are central to misfolded protein recognition and sorting to the various PQC compartments. For this, chaperones cooperate with other sorting factors and cellular structures such as the nuclear membrane, ER network, and cytoskeleton to recruit misfolded and damaged proteins to a particular quality control compartment (**Table 1**).

As chaperones also mediate de novo folding of newly synthesized proteins and the refolding of misfolded/aggregated proteins, an important question concerns how triage decisions, namely, to fold or sort to a PQC compartment, take place. Protein folding relies on several different ATP-dependent chaperone families, including the ring-shaped chaperonins and the Hsp70 and Hsp90 families (3, 6). Together with a plethora of cochaperones, these chaperones promote protein

folding and assembly through cycles of ATP-driven binding and release. Stress also upregulates members of these chaperone families, including Hsp70, Hsp90, Hsp110, and small heat shock proteins (sHSPs) (96), all of which have been implicated in PQC (6, 19, 109–112). Each of these heat shock protein families has also been shown to play a role in the formation of Q-bodies, the IPOD, the JUNQ and the INQ (21, 22, 32, 60). Hsp70s are central to all these pathways, but the selectivity for sorting of misfolded proteins to different compartments appears to be determined by their cochaperones.

Polypeptide substrate binding and release from Hsp70 is driven by cycles of ATP binding and hydrolysis, which are regulated by cochaperones such as the J-domain proteins and the nucleotide exchange factors (NEFs) (113, 114). The J-domain proteins, which promote substrate binding to Hsp70 as well as ATP hydrolysis, are themselves chaperones in their own right (113). Ydj1, Hlj1, and Sis1—all members of this family—are required for Q-body formation and maturation, with Sis1 shuttling being especially important for targeting of stress-denatured proteins to the nucleus and/or proteasomes (21, 23, 24, 115). Differential binding of the Ydj1 and Sis1 have also been shown to target prions to different fates (116). The Hsp70 nucleotide exchange factors Sse1/Hsp110, itself an ATP-dependent chaperone, and Fes1/HspB1 are also required for clearance and sorting to the Q-bodies (21, 117–119).

The HOOK family member Btn2 is another stress-induced protein implicated both in formation of the INQ and JUNQ and in targeting of prions (23, 60, 120, 121). In cooperation with Sis1, Btn2 is proposed to be a major sorting factor for nuclear deposition of aggregates (19, 23, 60). By contrast, interaction of Btn2 with the ATP-independent chaperone Hsp42 leads to the formation of peripheral inclusions (23). Hsp42 is an sHSP required for Q-body formation, but it also colocalizes with the IPOD under stress (21, 32). Under severe heat shock, another sHSP, Hsp26, becomes activated (122, 123) and colocalizes with spatial PQC sites (32). sHSPs are emerging as ATP-independent chaperones crucial for spatial sorting and sequestration of misfolded proteins. Organized in large oligomeric complexes, these proteins bind and suppress the aggregation of unfolded proteins, and their affinity for substrates is greatly increased upon heat shock. Interestingly, many sHSPs are regulated by the Daf-16/FOXO transcription factor (105, 124–126), which has been shown to stimulate a transcriptional induction of aggregases or sequestrases that promote formation of protective inclusions in worms (127).

Hsp26, together with the Hsp40–Hsp70 machinery, is also implicated in the disaggregation of heat-denatured proteins (128). This process is carried out by the yeast-specific AAA+ disaggregase Hsp104 (129, 130). In an ATP-driven process, Hsp104 forces aggregated polypeptides through its central pore; the unfolded polypeptide that emerges through the other side can then either refold spontaneously or as assisted by other chaperones (129). Hsp104 localizes to both the JUNQ and IPOD (21, 22), and its deletion prevents IPOD (but not JUNQ) formation and severely inhibits clearance of the JUNQ upon removal of stress (32, 60). There is no Hsp104 homolog in metazoans, likely because Hsp104 reduces cellular fitness under unstressed conditions (21). It is however proposed that chaperones such as Hsp110 and Hsp40 cochaperones might perform some of the same disaggregase functions (131–133). Of note, ubiquilins have recently been implicated in the clearance of aggregates by shuttling them to the proteasome for degradation (134).

Function of the Ubiquitin–Proteasome System in Spatial PQC

The UPS is responsible for the majority of misfolded protein clearance in eukaryotic cells (reviewed thoroughly in 135). In yeast, several E3 ubiquitin ligases have been implicated in the degradation of misfolded proteins, including Ubr1 or San1 in the cytosol or nucleus, respectively (117, 136–138), as well as the ER E3 ligases Hrd1 and Doa10 (139–142). Although the precise mechanisms

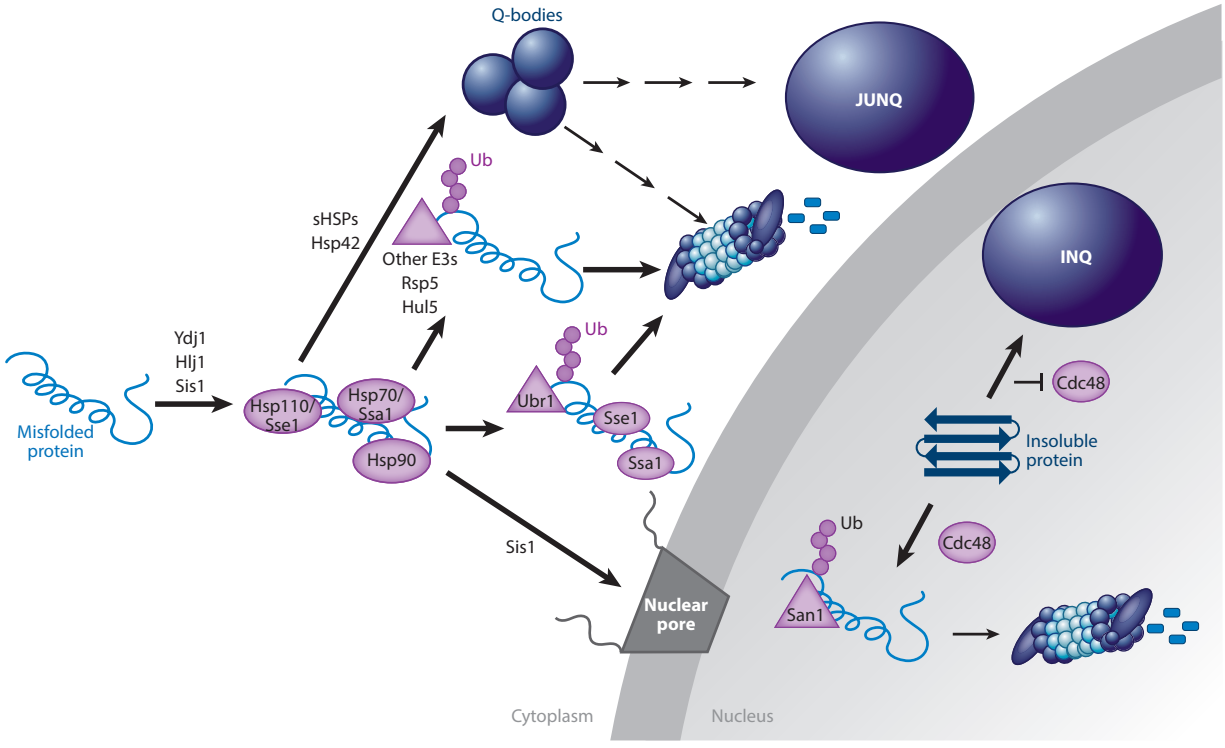

Figure 2

Multiple proteostasis pathways contribute to management of misfolded cytosolic proteins. A cytosolic misfolded protein that is bound by Ssa1/2 (Hsp70), Sse1 (Hsp110), and Hsp90 triggers a number of different protein quality control pathways. Binding of small heat shock proteins (sHSPs) leads to sequestration in Q-bodies en route to the juxtanuclear quality control compartment (JUNQ) or degradation by the proteasome. Ubiquitination by Ubr1 or other E3 ligases such as Rsp5 or Hul5 can also mark the protein for degradation by the proteasome. The ubiquitinated protein can be delivered by Sis1 to a site in or around the nucleus for proteasomal degradation. If Sis1 function is compromised (e.g., when its expression is lowered, its Hsp70 interaction domain is mutated, or it is sequestered to polyQ-expanded proteins in the insoluble protein deposit), the ubiquitinated protein accumulates in cytosolic puncta. The type I Hsp40 Ydj1 may also be required in this process. If ubiquitination by Ubr1 is blocked, some misfolded proteins can instead be transported to the nucleus—through a pathway requiring Sse1—to be ubiquitinated by San1. The AAA+ ATPase Cdc48 can also guide insoluble nuclear proteins to be ubiquitinated by San1, and blocking Cdc48 leads to sequestration of such proteins in the intranuclear quality control compartment (INQ).

of triage remain unknown, these E3 ligases are proposed to function in concert with chaperones, as schematized in **Figure 2** for Ubr1/San1 substrates (24, 59, 60, 115, 117, 138, 143).

A role for ubiquitin in targeting of proteins to the JUNQ was suggested by findings that (*a*) ubiquitin colocalized with the JUNQ; (*b*) proteasome inhibition enhanced JUNQ formation and impeded its clearance; and (*c*) overexpression of the deubiquitinating enzyme Ubp4p, or deletion of the E2 ubiquitin-conjugating enzymes Ubc4p and Ubc5p, blocked JUNQ formation (22). Furthermore, fusion of a single ubiquitin moiety to the yeast prion Rnq1 triggered its partial relocalization from the IPOD to the JUNQ, suggesting that ubiquitination itself was a sufficient sorting signal for spatial PQC. However, a more recent study (60) did not detect ubiquitin colocalization with VHL in the JUNQ or INQ. Although it is possible that substrate ubiquitination is not essential for JUNQ/INQ sorting, it is also possible that substrate-specific differences exist, which may be linked to the intrinsic solubility of the substrates (see the next section). Alternatively, it is

possible that the ubiquitin modification leading to JUNQ sorting is not a canonical K48-linked proteasomal targeting chain. Indeed, the finding that mono-ubiquitinated Rnq1 can localize to the JUNQ (22) supports this hypothesis.

Role of Autophagy Machinery Receptors in Clearance of Spatial PQC Sites

Autophagy was initially characterized as a mechanism for the bulk recycling of larger structures within nutrient-starved cells. However, it is becoming increasingly apparent that autophagy also plays a role in PQC networks, including the degradation of aggregated proteins (144). The autophagic clearance of aggregates, termed aggrephagy, involves the recognition of ubiquitin-labeled cargoes by a number of adapter molecules, such as HDAC6, BAG3, p62/SQSTM1, NBR1, Atg8, and Cue5 (144–146). HDAC6- and BAG3- mediated autophagy differ in the initial substrate-recognition steps, with Hsp70 recruitment to aggregates being required for the BAG3-mediated pathway (145). Additionally, HDAC6-mediated recognition requires K63-linked ubiquitination of aggregates, whereas recognition by BAG3 appears to be ubiquitin independent. However, both routes require the subsequent action of p62 and NBR1 for lysosomal degradation. Mammalian aggresomes containing amyloidogenic proteins appear to be bona fide substrates of autophagy (144, 147, 148).

Function of Spatial PQC Compartments: Refolding, Sequestration, or Degradation?

It is unclear what happens to misfolded proteins once they are sequestered in spatial PQC sites. They could remain there, become refolded by chaperones, or be cleared via the UPS or autophagy. It is likely that all three paths are possible, depending on the properties of the misfolded proteins and the cellular context.

The idea that solubility could be a key determinant of JUNQ and IPOD targeting comes from the finding that the proteins contained within the JUNQ and IPOD differ greatly in their mobility, as measured by FRAP (fluorescence recovery after photobleaching) and FLIP (fluorescence loss in photobleaching) assays (22). In the nucleus, aggregation-prone insoluble substrates require Cdc48 to maintain solubility so that they can be ubiquitinated by San1 (149). Cdc48/p97, an AAA+ ATPase best known for its role in extracting ERAD substrates from the ER for targeting to the UPS, may also play a similar role in the cytosol; that is, if a misfolded protein that would normally be ubiquitinated and degraded by the UPS cannot maintain solubility long enough for E3 ubiquitin ligase binding and/or ubiquitin transfer, the misfolded protein will aggregate and therefore become sequestered in the IPOD. Hsp70 may also play a role in maintaining solubility of ubiquitinated proteins that would otherwise be sorted to the IPOD in an Hsp42-dependent manner (115, 117).

Even if protein aggregates are cleared, for example, as observed upon removal of stress, it is difficult to determine whether they are refolded or degraded. It is likely that different types of aggregates have different fates. For example, disaggregation of ubiquitinated proteins by Cdc48/VCP is likely to release them for proteasomal targeting (149–151), although it should be noted that ubiquitin-mediated autophagy might also play a role in clearing these aggregates (152). Consistent with this notion, Cdc48/VCP is required for the autophagic clearance of stress granules in both yeast and mammalian cells (47). By contrast, Hsp104-triggered disaggregation probably favors refolding by the Hsp70–Hsp90 system (56, 129, 130). Note, however, that this may not hold true for all contexts, given that Hsp104 overexpression in aged yeast cells restores their otherwise impaired proteasomal activity (153), although it is possible that this is due to the release

of trapped proteasomes from aggregates rather than the proteasomal degradation of the proteins disaggregated by Hsp104.

Whether or not aggregated proteins that cannot refold are cleared through the UPS or autophagy is likely dependent on cell context. The relative contribution of the UPS and autophagy in protein degradation varies greatly in different cell types (154). Autophagy is thought to act as a back-up mechanism when the UPS cannot meet the cellular proteolytic burden, such as during stress (33, 155), perhaps because depletion of ATP reserves would inhibit several steps of the ubiquitination cascade and proteasomal activity. Furthermore, the UPS and autophagy have several overlapping elements (156), including the mammalian Hsp70 cochaperones CHIP and the BAG family, which might directly determine which system a protein is targeted to (157, 158). Consistent with this hypothesis, several aggregation-prone proteins can be degraded by either pathway (33, 159, 160).

Stress can also change the specificity of the UPS itself. One study shows that the E3 ubiquitin ligase Rsp5—which plays a key role in protein trafficking by catalyzing K63-linked substrate ubiquitination under physiological conditions in yeast—switches to a prodegradative enzyme that catalyzes K48-linked ubiquitination of a wide range of substrates upon heat shock (161). Rsp5 is also required for Cue5-dependent autophagy of polyQ-expanded Huntingtin upon nutrient starvation (146). Similarly, the E3 ubiquitin ligase Hul5, which is required to maintain cell fitness upon heat shock, increases proteasomal processivity and is required for ubiquitination of cytosolic proteins that are short lived or of low solubility (162). Therefore, stress has pleiotropic effects on cellular PQC, including changes in the chaperone machinery, upregulation of autophagic clearance, and remodeling of the UPS to meet changing cellular proteostasis requirements.

Managing Prions and Amyloid Proteins

Whereas PQC sites containing misfolded proteins are generally cleared upon relief from environmental stress, prion or amyloid protein aggregates tend to persist within cells. These aggregates tend to be sequestered in the IPOD (22, 36)—although one study reports that prions can form mobile compartments similar to the JUNQ under certain conditions (163).

It is thought that amyloid or prion aggregates are not suitable substrates for the UPS. Given that the IPOD colocalizes with the macroautophagy marker Atg8 and is located adjacent to the preautophagosomal structure, it is hypothesized that this compartment can be turned over by autophagy (22), although direct evidence for this has yet to emerge. However, the finding that the IPOD is generally inherited by mother cells during cell division (discussed in the next section) indicates that such aggregates are not effectively cleared by the cell and are instead sites of terminal aggregate sequestration. In fact, the disaggregation of prions by Hsp104 is a critical step in yeast prion propagation and toxicity (129, 164 165). It should be noted that the sequestration of prions in the IPOD can also contribute to proteotoxicity. For example, the Lindquist group found that toxicity of the yeast prion Rnq1 is linked to selective Rnq1-mediated sequestration of the spindle pole body protein Spc2 in the IPOD, thereby causing cell cycle arrest (166). Likewise, studies in mammalian systems have proposed that some aggregates (but not others) can sequester essential cellular proteins in sufficient amounts to produce a deleterious phenotype (167).

CONSEQUENCES OF SPATIAL PQC

The ultimate goal of all quality control systems—not just in biology—is to ensure that the released product is at a functional standard that is competent and suitable to fulfill its purpose. Release of a faulty product can have far-reaching consequences. In the context of a cell, release of faulty proteins

into the cellular milieu is directly linked to many diseases, including aging-related disorders, neurodegenerative conditions, metabolic disorders, and all types of cancer. It is unsurprising, therefore, that eukaryotic organisms have evolved such intricate systems of quality control. In this section, we describe how these PQC mechanisms keep the cell functional and viable and discuss how their malfunction is linked to disease onset and/or progression.

Quarantine of Potentially Toxic Species

Protein misfolding and amyloid aggregation is a hallmark of many aging-related neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases as well as ALS and many others. Additionally, protein misfolding can be linked to non-neuronal diseases, including lysosomal storage disorders, cystic fibrosis, cancer, and aging. These aggregates have been classified into two broad categories: those with β-sheet-rich amyloid and those without a regular structure, often called amorphous nonamyloid aggregates, which are sorted to distinct PQC compartments. The link between these aggregates and toxicity is unclear. It has been suggested that aggregates are protective and respond to the above-described pathways to sequester misfolded proteins (168, 169). In this view, it would be the dysfunction of protein sorting to compartments and the increase in misfolded soluble species in the cytosol that cause cellular toxicity (170). Alternatively, some studies have shown that protein aggregates are linked to toxicity, either outright or by aberrantly sequestering proteins necessary for cellular functions, such as chaperones and critical enzymes (10, 171). This might indicate that a certain type of aggregate or PQC compartment is linked to toxicity and other aggregates/compartments could be protective.

Compromising the sequestration of numerous nonamyloid, nonprion proteins has been shown to decrease cell viability. To illustrate with one example, depleting cells of Hsp90 results in the relocalization of several proteins of the galactose utilization pathway into Hsp104-positive inclusions (172). As imbalances in this pathway are known to cause fatal growth arrest due to the accumulation of toxic galactose metabolic intermediates, it is likely that this response to Hsp90 inhibition has a cytoprotective function. It is easy to imagine other similar scenarios in which spatial PQC acts as a general "mop-up" mechanism to correct perturbations in vital signal transduction networks. However, these pathways may also lead to pathological consequences. For instance, tumor-causing mutations in p53 cause it to aggregate in nonamyloid inclusions (101, 173). As p53 forms oligomers, the mutant p53 tends to drag the wild-type allele to these inclusions, exacerbating the loss of function (101, 173, 174).

Maintaining the Integrity of the Proteome

At the most fundamental level, spatial PQC serves to prevent imbalances in the proteome that can compromise cell function. In addition to sequestration of potentially toxic proteins that interfere with essential cellular processes, this also involves a number of mechanisms that alter synthesis and turnover of the proteome as a whole.

Stress granules and translational fidelity. Stress granules ensure that new proteins are not synthesized under conditions of stress, and allow for the rapid resumption of translation upon relief from stress. Due to the vulnerability of newly made proteins to misfolding, a halt in protein synthesis is presumably beneficial and also reduces the load on cellular proteostasis, allowing the cell to manage the increased formation of aggregates (51, 97, 175). Newly translated proteins may aggregate before they have a chance to reach their native conformation, because either the kinetic landscape of the stressed cell favors pathways leading to aggregation (175) or the existing

aggregates "seed" recruitment of normally non-aggregation-prone proteins, as described for one mechanism of prion propagation (176). Formation of stress granules increases cell viability (177) and can also delay entry into apoptosis (178, 179). Consistent with these findings, several studies have linked survival of cancer cells to stress granule formation in response to chemotherapeutic agents (180).

Whether stress granules have a protective or pathogenic role in the onset and progression of other diseases is a topic of controversy. A number of neurodegenerative disease-related proteins, such as TDP-43, Tau, Fus, Huntingtin, and PrP, are found associated with stress granules (181, 182). Many RNA-binding proteins found within stress granules possess low-complexity, aggregation-prone domains such as those found in prions (52, 183). An attractive hypothesis is that some of these disease-causing mutants, or stress, reduce the dynamics of stress granules; the stabilized stress granules could then drive the formation and propagation of pathogenic protein aggregates (181, 182). Viral infection also triggers robust stress granule formation, but again, whether or not they are beneficial to the host is unclear and may differ from virus to virus (184, 185).

Proteasome disassembly and relocalization in quiescent yeast. Rapidly dividing cells require constant cycles of protein synthesis and degradation (186). By contrast, quiescence (e.g., by nutrient starvation) leads to a drastic reduction in both these processes, presumably because the cell no longer needs to amplify its bulk protein level (187).

In yeast, starvation or stationary phase causes the 26S proteasomes to disassemble into 19S and 20S subunits (188) and form dynamic cytoplasmic puncta known as proteasome storage granules (PSGs) (189). Preventing PSG formation is linked to reduced viability in quiescent yeast cells (190). Note also that dysfunctional proteasomes can at this stage be removed from PSGs by association with the IPOD—a process dependent on Hsp42 and Cue5 (191, 192). Upon reentry into the cell cycle, proteasomes within PSGs reassemble and translocate back to the nucleus. Therefore, PSGs likely represent an inactive reservoir that not only prevents excessive protein turnover in quiescent phase but also allows rapid upregulation of degradation when log-phase growth resumes. It is unclear whether or not PSGs (or equivalent structures) form under conditions of stress. Given that stress granules have been shown to form upon quiescence (193), it would be unsurprising to find that PSGs exist in stressed cells. In this way, these two structures might provide a common means by which a cell can carefully control proteostasis under differing protein loads. Remarkably, other cellular complexes and enzymes localize to cytoplasmic storage granules in quiescent cells, many of which also contain Hsp42 (191, 192, 194–196).

Mitochondria as gauges of cellular function. Mitochondrial function might act as a sensor for the state of cellular function as a whole. This is not surprising given the importance of mitochondria in maintaining correct cellular energy levels. Indeed, severe mitochondrial dysfunction leads to cell death (90). Several mechanisms exist to delay this fate. We have already discussed how defective mitochondrial protein import causes the toxic accumulation of mitochondrial proteins in the cytosol, and considered the recent identification of a cytosolic response that alleviates this toxicity by reducing protein translation and increasing proteasome activity (91, 92). Accumulation of misfolded proteins inside the mitochondria themselves triggers the mitochondrial UPR, which upregulates mitochondrial chaperones and proteases and arrests mitochondrial protein translation (90, 197), analogous to the effects of cytosol-mislocalized mitochondrial proteins, and the UPR in the ER. Another recently discovered mechanism in aged yeast cells involves selective removal and autophagic clearance of part of the damaged mitochondria's membranes (and with it, any embedded proteins) but leaves the rest of the organelle intact (198). Should all else fail, the entire mitochondrion can be cleared by autophagy (mitophagy) (199). Given that mitochondrial

dysfunction is a hallmark of aging, cancer, and numerous neurodegenerative diseases (200, 201), it is likely that these quality control mechanisms play a key role in maintaining cell health—and may point to future avenues for therapeutic intervention. For example, artificially increasing mitophagy in human fibroblasts and whole mice was recently shown to dramatically reduce many of the deleterious effects associated with senescence-related aging (202).

Coping with PQC Decline upon Aging

A decline in mitochondrial function is just one of several intracellular changes observed during aging. In addition to increased formation of protein aggregates (105, 106), one of the best-characterized consequences of aging is the buildup of reactive oxygen species and, consequently, oxidatively damaged proteins (203). One damage-induced modification, carbonylation, normally marks proteins for degradation but can also lead to formation of high-molecular-weight aggregates (204, 205). Carbonylated aggregates have been implicated in a wide range of aging-related diseases. This, together with the increased accumulation of misfolded proteins, means that the burden on the PQC system is dramatically increased in aged cells.

Proteostasis has been shown to decline during aging in many eukaryotes (206, 207). It is unclear whether the components of the PQC systems themselves decline (e.g., a reduction in the capacity of molecular chaperones or the UPS to deal with misfolded proteins) or the capacity of these systems is simply exceeded by an increase in misfolded protein load (6, 207). In *Caenorhabditis elegans*, it seems that proteostasis failure is linked to a severe dampening of the heat shock response and the UPR (208). In humans, one study found that the expression of genes encoding ATP-dependent chaperones was decreased and that of ATP-independent chaperones was increased in aged brains (209). Strikingly, this same expression pattern was amplified in the brains of patients with neurodegenerative disorders. Additionally, PSG formation—which is linked to maintaining cell viability in quiescent yeast cells, as described above—is also reduced in aged yeast, and deletion of certain *N*-acetylation complexes essential for proteasome relocalization and PSG formation decreases fitness in young cells (210).

If there is such a severe reduction in PQC capacity, it is possible that aggregates formed in aged cells and tissues are morphologically and functionally distinct from those induced by stress in younger cells. In young cells, aggregates are formed actively by PQC systems to maintain cellular proteostasis. By contrast, aggregates accumulate in aged cells due to a failure or decline in these same PQC systems and so might contribute to pathogenesis. Consistent with this hypothesis, numerous groups have observed distinct differences between stress-induced and aging-related protein aggregates (29, 38, 211, 212).

Asymmetric Inheritance of Protein Aggregates

Although the mechanisms described above attempt to limit toxicity during the life span of a cell, other mechanisms exist to ensure that toxic species are not transferred to one of the daughter cells following cell division. Yeast cells provide aging paradigms for both mechanisms, whereby the chronological life span is defined as the length of time a single cell survives and the replicative life span as the number of divisions a cell can undergo (213). Yeast cells divide by asymmetric budding, with the daughter cell being smaller than the mother. Both the JUNQ and the IPOD are asymmetrically inherited by the mother cell during cell division in yeast (214).

Two important studies from the Nyström group identified that oxidatively damaged (27) and aggregated (28) proteins are almost exclusively sequestered in the mother cell during division. They identified the longevity-linked protein Sir2p as being critical for this process. Subsequent

studies have identified involvement of molecular chaperones (Hsp70, Hsp104, Hsp42, and the TRiC/CCT chaperonin), the actin cytoskeleton, the endocytic and vacuolar pathways, and proteins involved in tethering of aggregates to the ER or mitochondria (19, 214–218). Asymmetric inheritance would explain the full replicative potential of daughter cells, and also why mother cells—which accumulate protein aggregates and otherwise damaged proteins—show a limited replicative life span (219). Consistent with this hypothesis, abrogating asymmetric inheritance (e.g., in Sir2p mutants, by deleting *hsp42* or using actin depolymerization compounds) prolonged the replicative life span of the mother cell and reduced that of the daughter (29, 30). Overexpressing Hsp104p partially restored asymmetric inheritance and replicative fitness of the daughter cell. The importance of Hsp104p is further highlighted by the finding that its overexpression also restores proteasome activity in replicatively aged yeast, which suggests that UPS decline might be directly linked to aging-related accumulation of aggregates (153).

Asymmetric inheritance of protein aggregates is also seen during mitosis in mammalian cells, although the mechanisms are likely to be different and involve the intermediate filament vimentin (26) as well as attachment to the ER (220). Nevertheless, the presence of such a pathway in mammalian cells could be one mechanism by which replicative fitness is maintained in cells undergoing polar divisions, such as differentiating stem cells and tumors. Interestingly, the mechanisms to retain aggregates asymmetrically in dividing neural stem cells decline with age (220), which could in turn lead to impaired proteostasis. Of course, the molecular basis for aggregate toxicity is one of the most important questions that remains unanswered.

CONCLUSION

Spatial sequestration of misfolded proteins is a complex process that occurs in many locations in both yeast and mammalian cells. Although these processes are conserved, some of the compartments and pathways differ across cell types. Sequestration into PQC compartments is not tied to a breakdown in proteostasis or to disease but is an early physiological response of the cell to the presence of misfolded proteins. Much work is needed to understand the role these compartments play in the formation of both toxic and protective inclusions and what effect they have on cellular fitness and progression of diseases linked to aggregation. Understanding the basic cell biology involved in spatial sequestration may allow for the development of novel therapeutics for a wide range of diseases, from neurodegenerative disorders to cancer.

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