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Protein quality control in the nucleus

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The nucleus is the repository for the eukaryotic cell's genetic blueprint, which must be protected from harm to ensure survival. Multiple quality control (QC) pathways operate in the nucleus to maintain the integrity of the DNA, the fidelity of the DNA code during replication, its transcription into mRNA, and the functional structure of the proteins that are required for DNA maintenance, mRNA transcription, and other important nuclear processes. Although we understand a great deal about DNA and RNA QC mechanisms, we know far less about nuclear protein quality control (PQC) mechanisms despite that fact that many human diseases are causally linked to protein misfolding in the nucleus. In this review, we discuss what is known about nuclear PQC and we highlight new questions that have emerged from recent developments in nuclear PQC studies.

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Introduction

The misfolding of proteins is an unavoidable problem that every eukaryotic organelle encounters. Protein misfolding occurs via numerous mechanisms: genetic mutations, errors in transcription or translation, problems during nascent peptide folding, and stressors that damage structures of normally folded proteins. The devastating issue of protein misfolding is highlighted by the human pathologies that are causally linked to misfolded protein aggregation such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS) [1]. To prevent aggregation, eukaryotic cells have evolved robust and often interconnected compartmental protein quality control (PQC) pathways that manage misfolded proteins by either refolding them into functional proteins through chaperones [2], sequestering them into large inclusions via small heat shock proteins or chaperones [2], or degrading them through the ubiquitin-proteasome system [3] or autophagy [4,5] (Figure 1). From a historical view, much of what we have learned about eukaryotic PQC systems and their role in maintaining proteostasis has largely come from studies in the cytoplasm and endoplasmic reticulum (ER) [6]. By contrast, much less is known about PQC mechanisms in the nucleus. However, at least 15 human diseases are associated with misfolded protein aggregation in the nucleus and include Huntington's, many spinal cerebellar ataxias (SCAs), and spinal bulbar muscular atrophy (SBMA) [7].

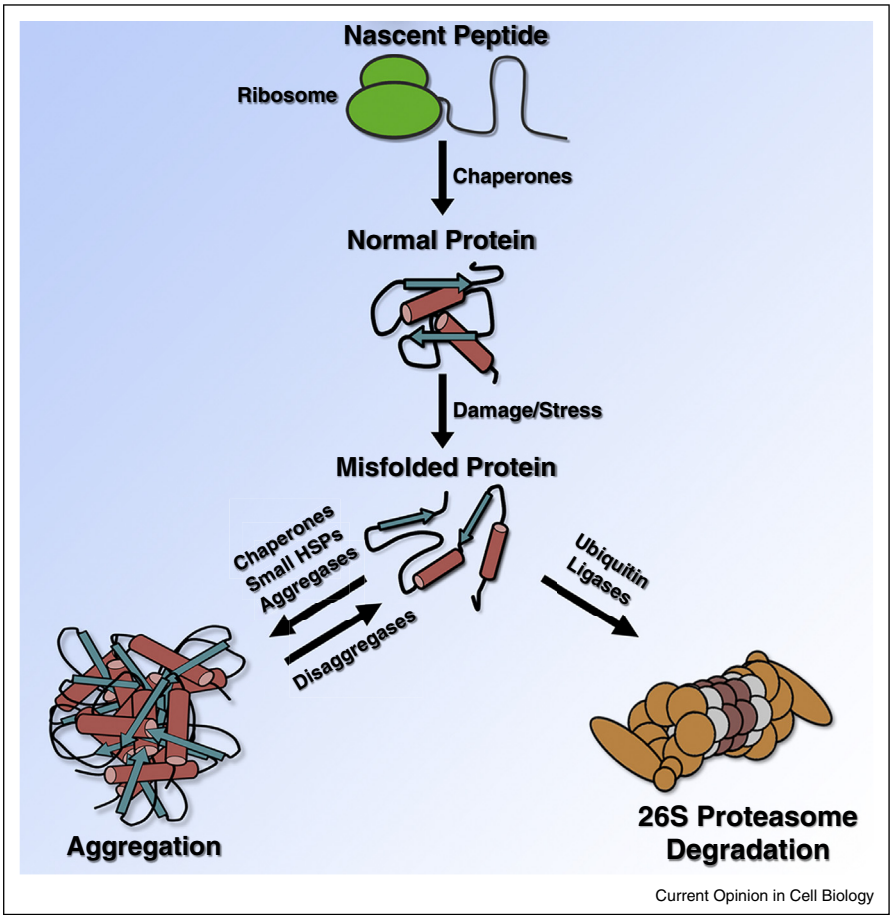
Overall nuclear protein quality control

Quality control studies in the nucleus have traditionally focused on how the cell maintains the integrity of the nuclear genome and the quality of mRNA before export from the nucleus [8,9]. By contrast, our understanding of how the cell maintains the quality of nuclear proteins has lagged behind. The protein aspect of overall nuclear quality control is exceptionally important to consider because an escalating failure to remove or repair misfolded nuclear proteins can lead to a deterioration in the integrity of the nuclear genome and the quality of the mRNA produced. This in turn can impact overall cellular proteostasis due to a reduction in the translational fidelity of the genetic code into its functional complement of proteins. Over the last decade, studies of misfolded nuclear proteins have revealed some of the folding and degradative PQC systems that function in the nucleus and these appear to operate on similar overarching principles as PQC systems in the cytoplasm with chaperones, small heat-shock proteins, and ubiquitin ligases generally managing misfolded nuclear proteins [10••,11••,12••,13••,14•,15•,16••,17••,18•,19••,20••,21].

One unique aspect of the nucleus is that it contains a high concentration of DNA, which is organized into chromatin. Because of the negative charge of DNA, many structural chromatin proteins have a considerable positive charge, such as histones. In addition, the nuclear proteome is enriched for proteins that possess low complexity, intrinsically disordered regions [22], suggesting these proteins have a broad capacity for conformational flexibility. Furthermore, chromatin is dynamic and there is ongoing remodeling that involves continuous assembly and disassembly of DNA–RNA–protein complexes [23]. These factors are potentially a considerable source for protein misfolding and aggregation specific to the nucleus.

Unlike the cytoplasm, one key PQC challenge the nucleus does not face to any considerable extent is the need to balance the robustness of PQC in the misfolding of damaged proteins with the PQC in the folding of nascent proteins. That is because nuclear proteins are synthesized

Figure 1



Overall summary of general PQC. General stages of PQC in the cell from nascent peptide synthesis to degradation.

in the cytoplasm and are imported into the nucleus through the nuclear pore [24]. Thus, nuclear PQC can be primarily focused on proteins that have become misfolded via damage during or after nuclear import, and nuclear PQC pathways may have evolved to target specific features of damage-induced misfolding that are particularly harmful in the nuclear environment. However, we note there is a possibility that the nucleus engages in limited translation [25], and may have to manage a low level of translational products. Furthermore, failures in cytoplasmic PQC can reduce the amount of correctly folded nuclear proteins, which can subsequently burden nuclear PQC pathways by decreasing the levels of functional nuclear proteins.

PQC degradation in the nucleus

The majority of proteasomes are nuclear localized [26], and the ubiquitin–proteasome system is the main route for misfolded protein degradation in the nucleus [3]. However, a recent study has suggested that nuclear proteins destined for proteasome degradation are first exported from the nucleus and destroyed by cytoplasmic proteasomes [27]. It is not clear if this is specific to a class of proteins that contain nuclear export signals or general for all nuclear proteins. However, it is clear that a number of nuclear-localized ubiquitin ligases have been implicated in the PQC degradation of misfolded nuclear proteins (Table 1). For this review, we will limit our discussion to the major yeast ubiquitin ligases involved in the degradation of misfolded nuclear proteins (Figure 2), as yeast has been the most extensively used organism for studies of nuclear PQC degradation.

The best understood yeast ubiquitin ligase involved in nuclear PQC degradation is San1. Originally discovered as a gene that, when mutated, suppressed the temperature-sensitive mutant phenotypes of *sir4-9* and *cdc68-1* cells [28,29], San1 was later found to be a ubiquitin ligase [10••,30] that is nucleoplasmic localized and specific in targeting misfolded nuclear proteins for ubiquitination and proteasome degradation [10••,12••]. Since the discovery that San1 is a nuclear PQC ubiquitin ligase [10••], additional genetic screens have revealed that loss of San1 leads to the stability of many other temperature-sensitive

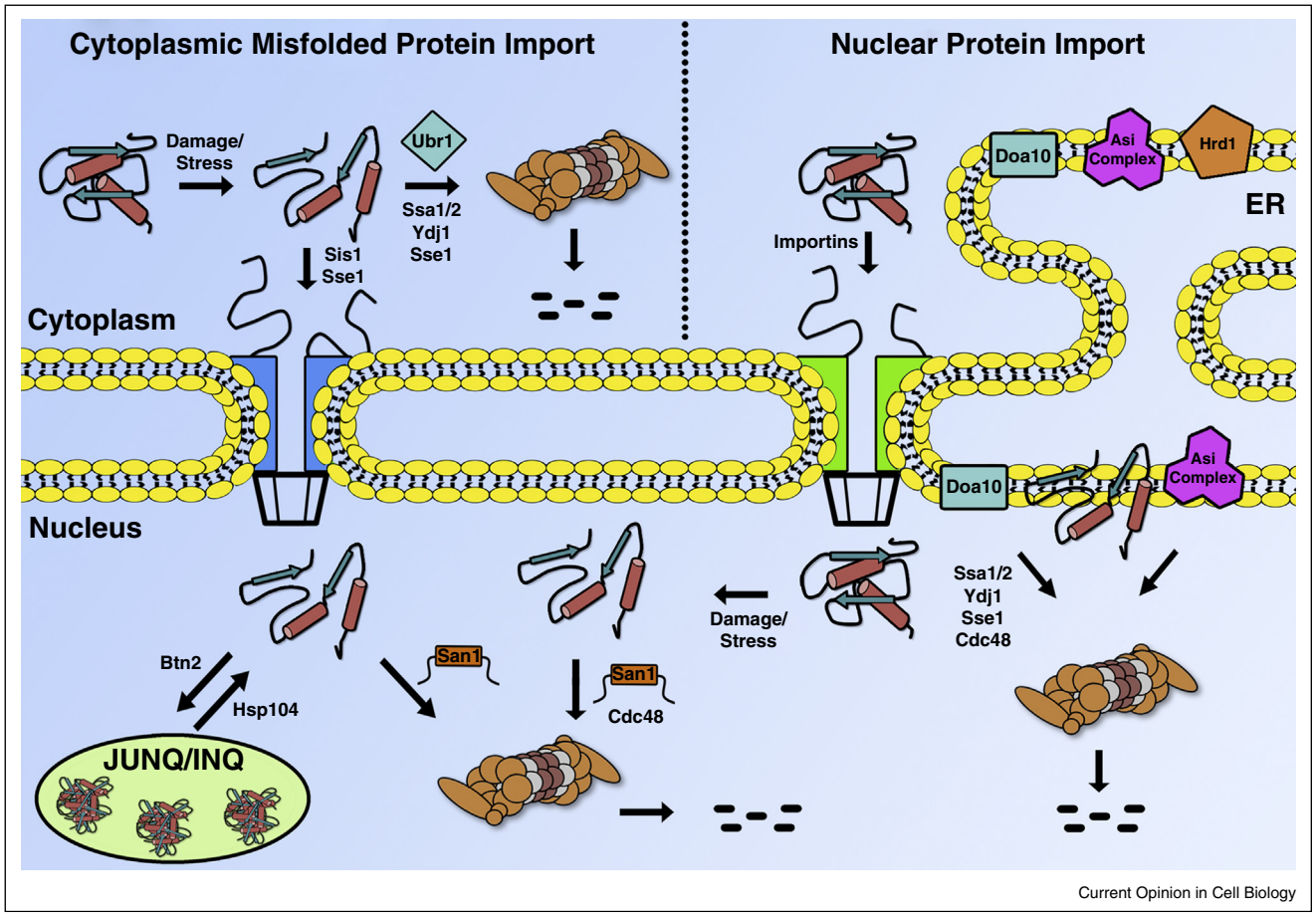
Table 1

Ubiquitin ligases implicated in nuclear PQC degradation

Ubiquitin ligase	Species studied	Localization	Key references
San1	Yeast	Nucleus	[10••,12••,30]
Ubr1	Yeast/mammals	Cytoplasm/nucleus?	[16••,17••,37••]
Doa10/Ssm4	Yeast	ER membrane/inner nuclear membrane	[19••,20••,46••]
Asi complex (Asi1, 2, 3)	Yeast	Inner nuclear membrane	[51••,52••]
Slx5-Slx8	Yeast	Nucleus	[41,99]
PML-IV/RNF4	Mammals	Nucleus	[71,100–103]
UHRF-1	Mammals	Nucleus	[104]
UHRF-2	Mammals	Nucleus	[104]

mutant nuclear proteins [31–34], indicating that degradation of a mutant nuclear protein is a common route to introduce temperature sensitivity. San1's ability to target misfolded nuclear proteins is attributed to large regions of intrinsic disorder within the N-terminal and C-terminal regions of San1 [12••]. These disordered regions possess multiple substrate-interaction regions within a conformationally plastic scaffold, which we propose provides San1 a broad mechanism to target many different kinds of misfolded proteins [12••,35]. San1 has been found to recognize exposed hydrophobicity within its misfolded substrates that would have normally been buried within the protein [11••]. San1 does not appear to favor a particular type of exposed hydrophobic residue; it only requires that the degree of total exposed hydrophobicity in its substrates is at a threshold that leads to insolubility and aggregation [13••], which also correlates in many cases with substrate toxicity [11••,13••]. San1 does have some

Figure 2



Overall summary of nuclear PQC degradation in yeast. Major ubiquitin ligases involved in nuclear PQC degradation in yeast are shown in their locations respective to the nuclear membrane. Chaperones involved in different aspects of nuclear PQC are also shown.

substrate overlap with the ubiquitin ligase Ubr1 [15•,16••,17••,18•], but Ubr1 most likely acts in the cytoplasm rather than the nucleus [16••,17••,36••,37••,38,39]. San1 has a homolog in fission yeast [40], but no functional equivalent has been identified in metazoans. This is likely due to the extreme intrinsic disorder of San1 [12••,35], which allows for a high degree of sequence divergence over evolution [41].

Residing in the ER/inner nuclear membrane, the ubiquitin ligase Doa10 has also been implicated in nuclear PQC degradation [19••,42•,43,44]. *DOA10* (aka *SSM4*) was first characterized as an mRNA quality control gene [45]. Mutation to *DOA10/SSM4* suppressed the temperature sensitivity of a mutant form of the nuclear Rna14 protein [45]. However, the Doa10 protein is best characterized for its role in ER-associated degradation (ERAD), ubiquitinating both soluble and ER-membrane substrates for degradation by the proteasome [18•,19••,20••,39,42•,46••,47–49]. Similar to San1, Doa10 also recognizes exposed hydrophobicity in its substrates, though the hydrophobicity differs from what San1 recognizes and appears to be presented as an amphipathic helix [19••,42•,43,46••,50]. The exact mechanism by which Doa10 binds its substrates still remains to be elucidated. Doa10 has a mammalian homolog called TEB4/MARCH-VI, but a PQC function has not yet been established for TEB4 [48].

In addition to Doa10, an inner nuclear membrane ubiquitin-ligase complex called Asi (composed of the yeast ubiquitin ligases Asi1 and Asi3, along with Asi2) has recently been implicated in the nuclear PQC degradation of misfolded inner nuclear membrane proteins and soluble transcription factors [51••,52••]. The Asi complex seems to function in a parallel pathway with the ER-membrane ubiquitin ligase Hrd1 to destroy misfolded nuclear membrane proteins [51••,52••]. However, more studies are needed to understand the broader role of the Asi complex in nuclear PQC, as well as its substrate specificity at the nuclear membrane.

PQC chaperones in the nucleus

A common characteristic amongst nuclear PQC ubiquitin ligases is that chaperones have been implicated in the degradation of some substrates [15•,16••,17••,19••,42•,44,53–55]. A few reports demonstrate that chaperones are required for the ubiquitination of misfolded cytoplasmic proteins destined for nuclear PQC degradation by San1 [15•,16••,17••]. Another study showed that ubiquitination via San1 does not universally require chaperones [12••]. This is a controversial aspect of nuclear PQC degradation because little is known about the precise role chaperones play in nuclear PQC degradation. In Table 2, we outline the chaperones implicated in nuclear PQC.

The linkage between chaperones and PQC degradation comes from a long history of PQC studies in the ER and cytoplasm [6], and has shaped how the PQC field thinks about the compartmental triage of misfolded proteins [56]. Ubr1 targets substrates in what appears to be a chaperone-dependent manner, with the Hsp70 chaperones Ssa1/Ssa2, the Hsp40 chaperone Ydj1, and the Hsp110 chaperone Sse1 required for Ubr1-dependent degradation [15•,16••,17••,18•]. Doa10 also targets substrates in what appears to be a chaperone-dependent manner, utilizing Ssa1/Ssa2, Ydj1, and the Hsp40 chaperone Sis1 [19••,42•,44,49,53–57]. Although the role of chaperones in San1-mediated degradation remains a subject of open debate [12••,15•,16••,17••,18•], the San1 pathway does utilize the AAA-ATPase chaperone Cdc48/p97 for the degradation of select San1 substrates that are highly insoluble [14•]. It may be that chaperone involvement in nuclear PQC degradation is important for partitioning misfolded proteins between insoluble aggregates and available monomers that can be recognized by the nuclear PQC degradation ubiquitin ligases. How chaperones function in nuclear PQC remains an open question. Both Sis1 and Sse1 are implicated in the nuclear transport of certain misfolded cytoplasmic proteins [16••,17••,58••], suggesting that one function for these chaperones may be to facilitate the trafficking of misfolded cytoplasmic proteins into the nucleus where San1 can recognize them.

Table 2

Protein chaperones implicated in nuclear PQC

Chaperone	Species studied	Localization	Key references
Ssa1/Ssa2 (Hsp70)	Yeast	Nucleus	[15•,16••,17••,18•,19••]
Ydj1 (Hsp40)	Yeast	Cytoplasm/nucleus	[15•,16••,17••,18•,19••]
Sse1 (Hsp70)	Yeast	Cytoplasm	[15•,16••,17••,18•]
Sis1 (Hsp40)	Yeast	Cytoplasm/nucleus	[53,58••]
Sti1	Yeast/mammals	Cytoplasm/nucleus	[105,106•]
Btn2	Yeast	Cytoplasm/nucleus	[63••,107•]
Hsp104	Yeast	Cytoplasm/nucleus	[91–93]
Hsp82/Hsc82 (Hsp90)	Yeast	Cytoplasm/nucleus	[39,94,106•]
Hsp26 (sHSP)	Yeast/mammals	Cytoplasm/nucleus	[95,108]
Hsp42 (sHSP)	Yeast	Cytoplasm	[63••]
Cdc48/p97	Yeast/mammals	Cytoplasm/nucleus	[14•,42•,109,110]

PQC inclusions in the nucleus

Although each cellular compartment possesses a robust complement of folding and degradative PQC activities, the burden of misfolded proteins can exceed the capacity of the compartment's PQC systems during stress or with age [59–61]. When the burden of misfolded proteins overwhelms a compartment's PQC pathways, misfolded proteins can aggregate [59–61]. One way the cell counters the incapacity of PQC pathways is to concentrate misfolded proteins into inclusion bodies that sequester aggregation-prone misfolded proteins. Prominent inclusions include the perinuclear mammalian aggresome [62], the perinuclear/intranuclear yeast JUNQ/INQ [63••,64••], and the perivacuolar yeast IPOD [64••].

The JUNQ/INQ is a primary nuclear inclusion for misfolded protein sequestration in both yeast and mammalian cells [63••,64••]. Originally identified as perinuclear [64••], a recent study found that the deposition is intranuclear and bounded by the nuclear membrane [63••]. Furthermore, genotoxic stressors like methyl methanesulfonate (MMS) appear to force important nuclear proteins into this inclusion [65••]. Interestingly, *san1*Δ cells were found to be sensitive to MMS in a large-scale genetic screen [66], suggesting that accumulation of misfolded San1 substrates could contributed to increased DNA damage. Alternatively, MMS can methylate Cys, His, Lys, and Arg residues in proteins [67], which could lead to protein misfolding and the need for San1 to remove MMS-damaged misfolded proteins.

The JUNQ/INQ is dynamic and the misfolded proteins in the JUNQ/INQ are mobile [64••]. However, the mobility of misfolded proteins in the JUNQ/INQ can decrease during stress [68,69]. The aggregase Btn2 is important for localizing misfolded proteins to the JUNQ/INQ [63••]. Furthermore, Hsp70 chaperones and proteasome subunits have also been shown to localize with the JUNQ [68]. Thus, the JUNQ/INQ is likely to be site where misfolded nuclear proteins are stored until they can be destroyed by ubiquitin-proteasome pathways. An important feature of the JUNQ/INQ is that localization of misfolded proteins to this site prevents their passage from the mother cell to the daughter cell during mitosis [70]. In this way, the quality of the nuclear environment in the daughter can be kept pristine.

In mammalian cells, nuclear inclusions have been studied using proteins with polyglutamine and/or polyalanine-expansions that lead to disorders such as Huntington's, Kennedy's, or oculopharyngeal muscular dystrophy (OPMD) [71–83]. In most cases, distinct chaperones have also been found to colocalize with the nuclear inclusions [71–83], similar to the JUNQ. It currently remains unknown if the nuclear inclusions in these cases are identical to the JUNQ or comprise different structures. Furthermore, different nuclear subcompartments may have their own unique sites of deposition. For example, an inclusion forms within the nucleolus after proteasome inhibition [84], though it is unknown if the nucleolar inclusion is composed of misfolded proteins. Much more work will be needed to understand the breadth and types of inclusions in the nucleus that act as PQC deposition sites.

PQC interplay between the nucleus and cytoplasm

Finally, it is important to consider that there will likely be significant interplay between nuclear and cytoplasmic PQC pathways as there is an intimate and dynamic communication between the two compartments through the nuclear pore. One interesting type of PQC interplay that has emerged in the last few years is that misfolded proteins are not always excluded from the nucleus if they are first generated in the cytoplasm [15•,16••,17••,18•]. This seems counterintuitive because cytoplasmic PQC pathways should prevent misfolded proteins from entering the nucleus where they can do harm. Why are these 'cytoplasmic' misfolded proteins imported into the nucleus when they should be managed in the cytoplasm? There are two possibilities. First, it could be that some misfolded cytoplasmic proteins are below the ~40 kDa passive diffusion limit of the nuclear pore [24]. This is the case for a number of misfolded cytoplasmic proteins studied [15•,16••,17••,18•]. Second, it could be there are active mechanisms to import misfolded cytoplasmic proteins to the nucleus if nuclear PQC degradation is more robust than cytoplasmic PQC degradation. Consistent with active import, it has been shown that the Hsp40 chaperone Sis1 is required for the nuclear localization and degradation of a misfolded cytoplasmic protein in yeast [58••]. In addition, deletion of the small heat shock protein Hsp42 leads to the nuclear localization of misfolded cytoplasmic proteins [63••], indicating that there are also retention mechanisms in the cytoplasm to prevent nuclear import. How transport and retention pathways coordinate to allow or disallow nuclear import of misfolded proteins is a new frontier for nuclear PQC studies.

Another possibility for interplay between cytoplasmic and nuclear PQC systems is that there may be conditions where cytoplasmic PQC pathways become overloaded and unable to buffer the cytoplasm against misfolded protein accumulation. For example, it has been shown that expression of toxic polyQ proteins in worms causes loss of function for chaperone-dependent, metastable proteins [85]. In addition, protein aggregation in the cytoplasm can interfere with nucleocytoplasmic transport [86]. It is possible that the nucleus serves as a back-up PQC compartment for cytoplasmic PQC when the cytoplasm cannot handle a high burden of misfolded proteins.

Lastly, some nuclear pore proteins are exceptionally long-lived in non-dividing cells [87,88], and nuclear pores have

been found to deteriorate with age, potentially allowing larger proteins to passively enter the nucleus [89•]. Thus, nuclear PQC pathways might become increasingly important with age as nuclear pore selectivity breaks down. This has significant ramifications for human health because at least 15 diseases have the common characteristic that they show greater level of misfolded protein aggregation in the nucleus that increases with age, and many of these proteins are not found in the nucleus at youthful ages [7].

Concluding remarks

We have gained foundational knowledge about nuclear PQC during the last decade. However, important pieces are still missing from the nuclear PQC puzzle. For example, it is not yet known if there is a mammalian equivalent to yeast San1. In addition, nuclear PQC functions for the mammalian homologs of yeast Doa10 and Ubr1 have yet to be shown. It will also be crucial to determine the particular features of misfolded proteins that are recognized by individual nuclear PQC degradation pathways, and how the pathways bind these features. Understanding the features of misfolding will also help delineate why chaperones are required for the nuclear PQC degradation of some substrates but not others. From a more holistic view, it will be important to explore nuclear PQC as a network and discover how the nucleus regulates the levels of its PQC systems in response to the burden of misfolded proteins. In the ER, the unfolded protein response (UPR) is crucial for protecting the ER environment during stress [90]. Evidence that a nuclear UPR might exist comes from the increased expression of nuclear chaperone and stress response genes when San1 function is compromised [10••]. Particular chaperone genes include those that encode for Ssa4, Hsp26, and Hsp104, all of which become enriched in the nucleus upon exposure of cells to protein misfolding stressors [91–98]. Revealing how nuclear PQC systems are coordinately regulated to protect the nuclear environment will be essential to understanding how these systems might fail during aging and lead to nuclear aggregation diseases.

Acknowledgements

We tried our best to cite all primary literature pertaining specifically to nuclear PQC. We apologize to our colleagues if we unintentionally missed their relevant studies due to space constrictions. This work was supported by a NIH/NIGMS training Grant 5T32 GM007750 to R.D.J. and a NIH/NIA Grant R01 AG031136 to R.G.G.

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