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Advances in nuclear proteostasis of metazoans

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ABSTRACT

The proteostasis network and associated protein quality control (PQC) mechanisms ensure proteome functionality and are essential for cell survival. A distinctive feature of eukaryotic cells is their high degree of compartmentalization, requiring specific and adapted proteostasis networks for each compartment. The nucleus, essential for maintaining the integrity of genetic information and gene transcription, is one such compartment. While PQC mechanisms have been investigated for decades in the cytoplasm and the endoplasmic reticulum, our knowledge of nuclear PQC pathways is only emerging. Recent developments in the field have underscored the importance of spatially managing aberrant proteins within the nucleus. Upon proteotoxic stress, misfolded proteins and PQC effectors accumulate in various nuclear membrane-less organelles. Beyond bringing together effectors and substrates, the biophysical properties of these organelles allow novel PQC functions. In this review, we explore the specificity of the nuclear compartment, the effectors of the nuclear proteostasis network, and the PQC roles of nuclear membrane-less organelles in metazoans.

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Abbreviations

| | |
|------|--|
| PQC | Protein Quality Control |
| PN | Proteostasis Network |
| MLO | Membrane-Less Organelle |
| NPC | Nuclear Pore Complexes |
| IDR | Intrinsically Disordered Region |
| IDP | Intrinsically Disordered Protein |
| LLPS | Liquid-Liquid Phase Separation |
| PML | ProMyelocytic Leukemia |
| GC | Granular Component |
| NLS | Nuclear Localization Sequence |
| UPS | Ubiquitin-Proteasome System |
| IPOD | Insoluble PrOtein Deposit |
| JUNQ | JUxtaNuclear Quality control compartment |
| INQ | IntraNuclear Quality control site |
| DRiP | Defective Ribosomal Product |

1. Introduction: proteostasis network and protein quality control at a glance

Proteins, essential macromolecules crucial for cellular function, face the risk of misfolding, posing a threat to cell viability. To counteract this, cells across all life kingdoms deploy a network of protein quality control (PQC) mechanisms to reduce and neutralize aberrant proteins under diverse stress conditions (Fig. 1) [1–4]. The accumulation of misfolded proteins is linked to aging and human pathologies like cancer, type II diabetes, and neurodegenerative disorders [5,6], emphasizing the need to comprehend pathways dealing with misfolded proteins.

Protein homeostasis or proteostasis - the state of proteome balance - is achieved by the collective and concerted action of a large array of components known as the proteostasis network (PN) (Fig. 1) [4,7]. The PN encompasses various activities including initial protein folding and conformational maintenance mostly undertaken by molecular chaperones, protein biogenesis mediated by the ribosome, and clearance of aberrant protein species by the cellular proteolytic machinery, including the autophagy pathway and the ubiquitin-proteasome mediated degradation. Components of the PN are heavily regulated upon proteotoxic stress conditions allowing the rapid and efficient repair of the proteotoxic stress-induced damages [8–10]. The complex architecture of eukaryotic

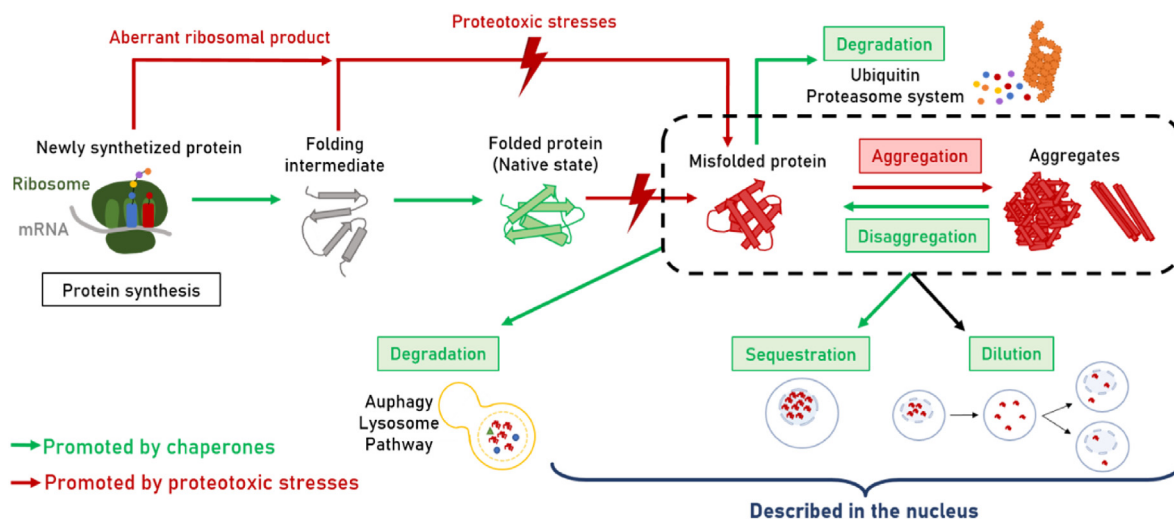


Fig. 1. The proteostasis network

A network of protein quality control effectors and strategies allows cells to maintain proteome integrity. Proteotoxic stresses promote the generation of aberrant proteins (red arrows), while a number of factors, including molecular chaperones, act to reverse this process by employing a diversity of strategies (green arrows). Steps identified in the nucleus are highlighted.

cells, with their assortment of membrane-bound and non-membrane-bound organelles, requires specialized PQC components. These components are precisely adapted to the unique proteomes and functions of each organelle, ensuring tailored and effective proteostasis throughout the cell.

Historically, much of what we know about eukaryotic PN and associated PQC pathways has largely come from studies focusing on the cytoplasm, the endoplasmic reticulum, and to a lesser extent the mitochondria [2,9,10]. By contrast, the strategies employed by the nucleus to address proteotoxic stress and manage aberrant proteins have only recently started to be unveiled. This is surprising given the number of essential processes occurring in this compartment such as DNA replication and repair, gene regulation, transcription, ribosome biogenesis, and mRNA splicing and editing. Quality control studies in the nucleus have traditionally focused on how the cell safeguards the genome, the transcriptome, the epigenetic marks among others [11]. However, our understanding of how the cell maintains the quality of the nuclear protein fraction has lagged behind while the nuclear proteome allows proper functioning of all nuclear processes. Nuclear protein misfolding is associated with a decreased integrity of genomic DNA and aberrant mRNAs [12]. Consequently, errors incorporated into the genome or transcriptome lead to the production of misfolded proteins, initiating a vicious cycle of escalating aberrant protein generation and proteome imbalance. The PQC machineries that protect the nucleus against misfolding may be either nuclear resident or imported from the cytoplasm as it has been observed decades ago [13–15].

The last decade has seen a revolution in cell biology with the new description of Membrane-Less Organelles (MLOs) as phase-separated compartments. MLOs include for instance P bodies or stress granules in the cytosol. The nucleus is remarkably rich in such MLOs that play integral roles in nuclear processes. Recently, some of these compartments have been reported to be involved in the management and processing of nuclear misfolded proteins. This parallels the emerging concept that cells are able to triage misfolded proteins into specific intracellular locations or into aggregated structures with different properties and fates [16]. The reasoning is that these structures are able to maintain aberrant proteins into a competent state for further refolding or degradation, or simply to protect cells from the toxicity of soluble species [17–21]. Some of these organelles can also concentrate PQC components and there are increasing number of evidence that molecular chaperones play a role in the maintenance and fate of these assemblies [22–24]. Moreover, some disease-related aggregation-prone proteins interfere with the biophysical properties and functions of nuclear MLOs [19,25–28]. The importance of understanding nuclear proteostasis and associated PQC mechanisms is highlighted by the fact that many conformational disorders are linked to nuclear protein misfolding and aggregation. They include p53-associated cancers and many neurodegenerative diseases such as polyglutamine-expanded diseases (e.g., Huntington's disease, the spinocerebellar ataxias), amyotrophic lateral sclerosis, frontotemporal dementias, and others [25–33].

In this review, we discuss the recent developments in the field of nuclear proteostasis and associated PQC in metazoans. In particular, we emphasize on the emerging role of nuclear MLOs in maintaining nuclear proteostasis and their PQC functions. The uniqueness of the nuclear environment is described first. We review PQC components identified as acting in the nucleus regardless whether they are nuclear resident or imported from the cytoplasm upon proteotoxic insult. Finally, we highlight new questions that have emerged from recent developments in nuclear PQC studies.

2. Unique features of the nuclear compartment

The nucleus is a distinctive feature of eukaryotic cells. It hosts the genetic information that must be packed, maintained, amplified, and transcribed, while translation occurs in the cytoplasm. This spatial segregation of crucial processes allows for a greater spatiotemporal control of gene expression and protein synthesis, safeguarding genetic information and transcription from the potentially interfering activities of the cytoplasm. Additionally, the nuclear physical barrier provides also space and time for the processing of RNAs such as splicing, 5' capping, and poly-A tailing, which are prerequisites for translation in the cytoplasm. The compartmental separation between transcription in the nucleus and translation in the cytoplasm contributes to the complexity and functionality of eukaryotic cells. After translation, most nuclear proteins enter the nucleus already folded protecting thereby the nucleus from translation and folding errors but exceptions exist such as defective ribosomal products that also accumulate within the nucleus [20]. Protein biogenesis is the most significant source of aberrant proteins as the decoding of mRNAs into polypeptide chains is prone to incorporate errors and protein folding is a fragile process with the risk of forming aberrant intra- and intermolecular interactions [1]. Translation within the nucleus has been reported but does not account for a significant fraction of the nuclear proteome and is still a highly debated topic [34]. Thus, the nuclear PN must be adapted for conformational repair and degradation of aberrant protein species rather than initial folding.

2.1. Nuclear transport

The nucleus is separated from the cytoplasm by the nuclear envelop, a double lipid bilayer membrane, extending from the endoplasmic reticulum, that separates the nuclear content from the cytoplasm (Fig. 2). Within the nucleus of metazoan cells, the nuclear envelop is lined with the nuclear lamina. This matrix of proteins is physically linked to the nuclear envelop. The nuclear lamina is a structural element of the organelle, but it is also involved in the regulation of gene expression as it binds to chromatin [35]. The nuclear lamina is essential for nuclear functions and any alteration of its proteins (lamins) is associated with a class of human diseases known as laminopathies including progeria [36]. The nuclear proteome is dependent on the integrity and permeability of the nuclear envelop and its Nuclear Pore Complexes (NPC). NPC are composed of more than 30 different proteins that need to be properly assembled into a large transport channel accommodating passively a globular protein of up to about 35 kDa [37]. Larger proteins translocate in the nucleus using energy-dependent transport, which involves the recognition of cargos by nuclear transport receptors using the GTP-bound state of the small G protein Ran, the importins and exportins. Importins bind to Nuclear Localization Sequence (NLS) within their cargos in the cytosol before translocation in the nucleus through the NPC. Once in the nucleoplasm, the importin binds to RanGTP triggering the release of the cargo protein. Nuclear export occurs in the opposite way [38]. Other transport systems using the RanGTP mechanism have been reported. A striking feature of the NPC is the presence of filaments within the pore lumen that contain phenylalanine-glycine repeats from nucleoporin proteins. These domains are aggregation-prone and are able to phase separate. This has been associated to their selectivity [39,40], mRNA export from the nucleus is also mediated by the NPC and several complexes [41]. The nucleocytoplasmic shuttling machinery is a target and dysregulated upon expression of various pathological aggregation-prone proteins [38].

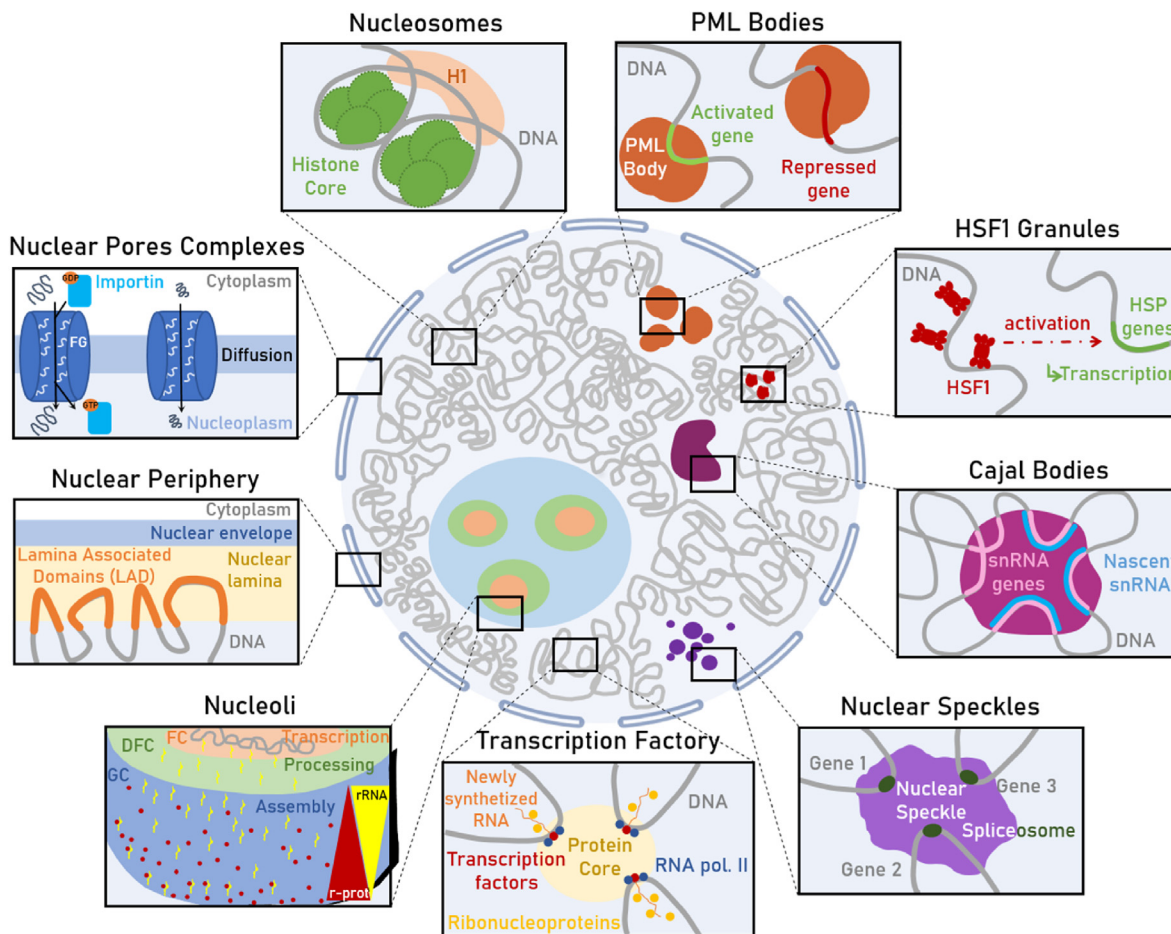


Fig. 2. Nuclear membrane-less organelles

The nucleus is physically separated from the rest of the cell by the nuclear envelope (light blue). Various nuclear membrane-less organelles and associated processes are highlighted (boxes).

Additionally, it is thought that the selectivity of the NPC declines during aging giving more opportunity to the cytoplasmic content to challenge the nuclear proteome and nuclear functions [42]. Thus, the correct selectivity and fidelity of the nuclear transport system is the first safety mechanism that preserves nuclear proteostasis. Interestingly, the importance of this nuclear barrier is highlighted by the fact that upon proteotoxic insult, the nucleocytoplasmic shuttling of proteins is largely attenuated while molecular chaperones such as Heat shock Protein 70 (HSP70) accumulate within the nucleus [43–45]. Thus, cytoplasmic misfolded proteins are virtually left within the cytoplasm and molecular chaperones are specifically imported for conformational repair of damaged proteins within the nucleus.

2.2. The nuclear protein fraction

A hallmark of the nuclear proteome is its abundant composition of Intrinsically Disordered Proteins (IDPs) and proteins featuring Intrinsically Disordered Regions (IDRs). Such proteins or regions are dynamic conformational ensembles of various conformers separated by low energy barriers [46]. Therefore, they are highly sensitive to environmental changes such as temperature, interaction with a partner, or post-translational modifications. This flexibility ensures the multifunctionality characterizing IDPs. IDRs are often of low complexity and are enriched in charged and polar amino acids and depleted in hydrophobic ones [46]. They usually bind to a large

number of partners via low affinity and high specificity interactions [47]. Moreover, their conformational adaptability make them prone to assemble/partition within the numerous nuclear MLOs, critical for nuclear function but also increasing the risk of misfolding. Consequently, the nuclear proteome is particularly vulnerable to proteotoxic damages, suggesting that effective surveillance and PQC ensure nuclear proteome functionality.

2.3. The nuclear membrane-less organelles

The nucleus itself is highly compartmentalized (Fig. 2). Its interior is organized into domains often highly associated with specific functions such as ribosome biogenesis, RNA processing and splicing, silent chromatin, and actively transcribed chromatin [48,49]. Some compartments, such as the nuclear stress bodies, can rapidly form in response to stimuli including stress signals such as DNA damage (UV, IR), chemotherapeutics, viral infection, oxidative stress [50,51]. These nuclear domains are MLOs and have been described as assemblies arising from at least Liquid-Liquid Phase Separation (LLPS) mechanism. They are also named biomolecular condensates (biocondensates) because of their formation through condensation of their constituents. The formation of these non-stoichiometric assemblies is linked to the property of polymers (IDP, RNA) to separate into a dense (enriched) and a dilute (depleted) phase under molecular supersaturation. This process can be controlled or altered by various environmental changes

including temperature, pH, ionic strength, and a number of other factors [52]. Multiple weak inter- and intramolecular interactions (electrostatic, π - π , and cation- π) of disordered polymers, primarily IDPs and RNA, play a central role in the formation of nuclear biocondensates as well as the regulation of their composition and functional activity [46,52]. Their composition usually involves protein, RNA, and DNA components. These nuclear biocondensates are usually associated with specific functions related to RNA metabolism and overall gene expression [48]. The absence of a membrane allows them to communicate with the dilute phase and to tune their compositions in a short time scale. However, under certain conditions, such bodies are able to form stable hydrogels and even insoluble aggregates. Indeed, a growing list of diseases and medical conditions including conformational disorders are associated with aberrant phase separation and transition [53].

Within the nucleus, the most prominent nuclear biocondensate is the nucleolus. It forms around ribosomal DNA and is the site for the coordinated assembly of ribosome subunits and signal recognition particles among other functions [51]. Ribosome biogenesis is a complex and delicate process of assembling in proper stoichiometry about 80 different ribosomal proteins and four ribosomal RNAs into two ribosomal particles and requires hundreds of ribosome biogenesis factors and small nucleolar RNAs [54]. Other large structures in the nucleus include the Cajal bodies, involved in ribosome biogenesis, telomeres regulation, among other functions. ProMyelocytic Leukemia (PML) bodies which are involved in multiple genome maintenance pathways and oxidative signaling are disrupted in promyelocytic leukemia [55,56]. These dynamic structures are directly or indirectly involved in nuclear PQC. Over the last decade, this metastable state of matter has also been observed to be vulnerable and sensitive to environmental changes.

3. Molecular chaperones in nuclear proteostasis: rewiring oldies

Biogenesis of nuclear proteins and initial folding occur in the cytoplasm before their import into the nucleus. With the nucleus hosting IDR-rich proteins and sensitive MLOs critical for its function but prone to aberrant interactions and aggregation (see part 2.2), the nuclear chaperone network (Table 1) plays a pivotal role in conformational surveillance and repair to maintain proteostasis in this compartment.

3.1. General features of molecular chaperones

The term “molecular chaperone” was first introduced in 1978 after purification of the histone chaperone nucleoplasmin [58]. Nucleoplasmin is an acidic protein that masks the positive charges of histones and prevents aberrant ionic interactions between DNA and histones. Molecular chaperones encompass diverse proteins from various families, including HSP100, HSP90, HSP70, HSP60, and small HSPs (sHSPs) [1,59]. Molecular chaperones recognize accessible or exposed hydrophobic protein patches, often buried within the native structure of the protein. Some chaperones utilize ATP energy to operate cycles of binding and release of the client proteins to promote folding. Chaperones such as sHSPs, also known as holdases, function without ATP hydrolysis [1,59]. Holdases preferentially bind (or “hold”) onto the unfolded state of proteins, thereby preserving their solubility, preventing aberrant off-pathway interactions, and inhibiting irreversible aggregation as a result. Chaperones are at the core of the PN as they sustain a number of PQC functions by interacting with various factors of other branches of the PN. Thus, beyond protein folding and repair, molecular

Table 1
Nuclear protein quality control components

| Component | Localization upon proteotoxic stress | Role in nuclear proteostasis | References |
|-----------------------|--|---|----------------------|
| HSP70 1A/B | Translocates into the nucleolus (GC), nuclear speckles | Refolds misfolded proteins in the GC phase of the nucleolus Protects unassembled ribosomal proteins Restores nucleolar morphology and functions Translocates misfolded proteins into the nucleolus | [19,21,90,93,94,138] |
| DNAJB1/6 | Translocates into the nucleolus (GC) | Solubilizes A-bodies and HSF1-stress bodies Resolubilization of nucleolar misfolded proteins Involved in delivering cytoplasmic misfolded proteins to the nucleus for degradation | [19,21,93,97] |
| NPM1/3 | Nucleolar (GC) | Interacts with misfolded proteins preserving them from irreversible aggregation (holdase) | [19] |
| NCL | Nucleolar (GC) | Interacts with misfolded proteins preserving them from irreversible aggregation (holdase) | [19] |
| DAXX | Nuclear | Protects nuclear proteins from misfolding and aggregation Acts as disaggregase and foldase in an ATP-independent manner | [70] |
| Proteasome 26S | Nuclear, p62 bodies, PML bodies, and nucleoli | Degradation of aberrant proteins | [19–21,78,120] |
| UBE20 | Nuclear | Degradation of orphan ribosomal proteins Holdase function | [108,126] |
| HUWE1 | Nuclear | Degradation of orphan ribosomal proteins Stress-induced neddylation of nucleolar-related inclusion proteins | [109,110,143] |
| RNF5 | Nuclear membrane | E3 ligase mediating degradation of aberrant nuclear membrane proteins | [113] |
| SYVN1 | Nuclear membrane | E3 ligase mediating degradation of aberrant nuclear membrane proteins | [113] |
| UBE2G2 | Nuclear membrane | E2 ligase mediating degradation of aberrant nuclear membrane proteins | [113] |
| SQSTM1/p62 | P62/SQSTM1 granules | Sequesters UPS machinery and substrates in nuclear biocondensates | [120] |
| PML/TRIM19 | PML bodies | Sumoylates nuclear aberrant proteins | [78] |
| RNF4 | PML bodies | Ubiquitylates sumoylated misfolded nuclear proteins | [78] |
| STUB1/CHIP | Translocates into the nucleolus | Involved in degradation of aberrant nuclear proteins Involved in the management of nucleolar misfolded proteins Acts as a holdase | [19,125] |
| NEDD8 | Nucleolar-related inclusion | As an hybrid chain with ubiquitin acts as an aggregase and precludes substrate degradation | [143] |

Repertoire of protein components with known roles in nuclear protein quality control. Known nuclear localization of components upon stress is given. Identified roles in nuclear protein quality control are given as well as important associated references.

chaperones are also involved in a plethora of other functions including disaggregation of protein aggregates and complexes, regulation of MLOs, and interact with the degradation machinery as well [22,24,60]. The function of chaperones is regulated by co-chaperones to enable interactions with other components of the PN, to control the rate of ATPase activity, and to provide substrate specificity [61–63].

3.2. Nuclear-resident chaperones

3.2.1. Guardians of histone proteostasis: the role of histone chaperones

The nucleus houses a specialized group of molecular chaperones, known for their involvement in chromatin dynamics: the histone chaperones. These crucial players are instrumental in overseeing all aspects of histones and nucleosomes proteostasis. Because of their crucial roles in escorting and remodeling nucleosomal and non-nucleosomal histones, histone chaperones control the accessibility and dynamicity of the chromatin. Mechanistically, all histone chaperones share the function of binding and shielding histone interfaces, maintaining them in non-nucleosomal conformations, and preserve them from off-pathway interactions. Histone chaperones, being a diverse class with low sequence and structural conservation, employ both folded domains and IDR in their histone-chaperoning tasks. HSP70 recently entered the field of histone chaperones through its interaction with DNAJC9 displaying an activity on histones H3–H4 [64]. The heterogeneity of histone chaperones is linked probably to the diversity of tasks they undertake. Indeed, beyond their role in nucleosomal context, these

chaperones provide essential buffering for the abundant histones, preventing their aggregation and fortuitous interactions with DNA. From synthesis at the ribosomes to assembly into nucleosomes, histone chaperones expertly guide histones through this intricate journey. Histone chaperones are also crucial for multiple other aspects of histone proteostasis including their storage, recycling, transport, post-translational modifications, remodeling, reassembly, and turnover. Therefore, the loss of histone chaperoning function has profound effects, including genomic instability, cell cycle arrest, DNA damage, and cell death. For more detailed information about histone chaperones in chromatin remodeling and gene expression, we direct readers to the comprehensive reviews and the references provided [65,66].

3.2.2. Histone chaperones as chaperones for non-histone proteins

Recent works have described chaperone functions of histone chaperones beyond histone proteins. Nucleophosmin 1 (NPM1) from the nucleoplamin family is a typical example that has been in the spotlight these recent years. In vitro, NPM1 is capable to work as holdase for various non-histones substrates and eventually also as foldase [67]. More recently, a structural study further elucidated the aggregation prevention mechanisms of NPM1 on amyloids, linked to its oligomerization capacity [68]. In vivo, the Granular Component (GC) phase of the nucleolus displays a holdase function [19,21]. This sub-nucleolar compartment is enriched with the abundant nucleolar NPM1 protein. In the GC, NPM1 and other similar proteins such as nucleolin (NCL) interact with misfolded proteins, preserving them from irreversible aggregation [19].

The histone chaperone DAXX (Death domain-associated protein

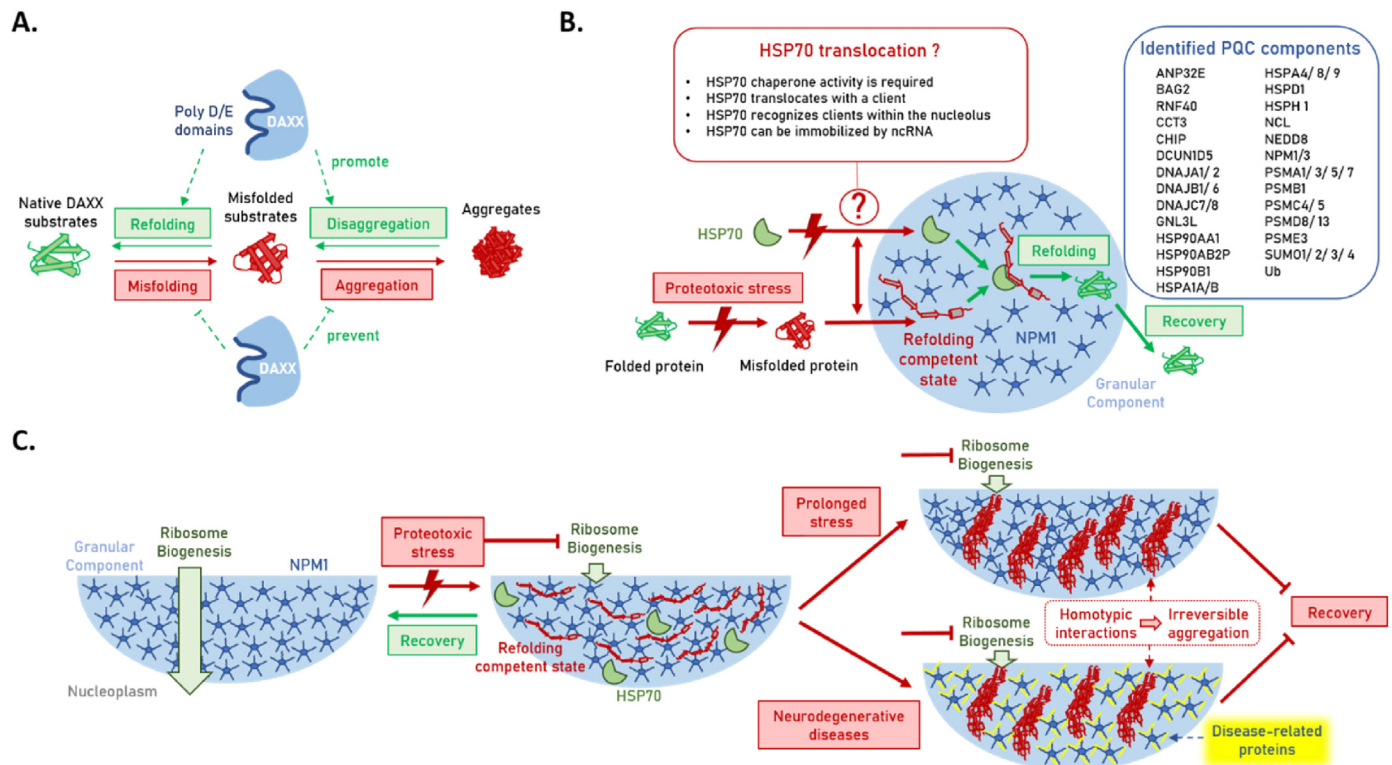


Fig. 3. Novel chaperone functions

A. DAXX functions as an ATP-independent multitasking molecular chaperone through its long charged poly-D/E region. **B.** The nucleolus functions as a phase-separated protein quality control compartment. Misfolded proteins immerse into the liquid-like granular component (GC, light blue) phase of the nucleolus, where they associate with nucleolar proteins (NPM1, dark blue). Upon recovery, misfolded proteins are refolded in an HSP70-dependent manner. Identified PQC effectors accumulating in the GC during heat-stress are shown (blue box). The proposed determinants of HSP70 translocation into the nucleolus are shown (orange box). **C.** The holdase capacity of the GC buffer excessive homotypic interactions between misfolded species. The PQC capacity of GC can be overwhelmed or altered in particular by disease-related proteins.

6) is known for its function on the histone H3.3 [69]. Recent evidences demonstrate that DAXX can protect proteins from unfolding (Fig. 3A). Even more remarkable is DAXX's capability to extract polypeptide chains from protein aggregates, effectively serving as a disaggregase. DAXX was also shown to be able to refold misfolded proteins but in an ATP-independent manner [70]. These diverse functions were attributed for the large part to an acidic amino acids (i.e., aspartates and glutamates) rich domain (poly-D/E). Other poly-D/E domain containing proteins could recapitulate some of these findings [70]. Remarkably, DAXX prevents and reverses aggregation of its client proteins, in particular the tumor suppressor p53 and its principal antagonist MDM2. Moreover, DAXX is able to refold and restore the function of known aggregation-prone p53 mutants thereby reducing their oncogenic properties (Fig. 3A) [70]. DAXX's unique recognition mechanism involves binding to exposed basic protein regions, distinguishing it from canonical molecular chaperones. While chaperones such as HSP70, bind to exposed hydrophobic residues that are otherwise buried within the structure of the native protein, DAXX targets misfolded clients with exposed basic regions. However, basic protein regions of folded proteins are usually solvent-exposed raising the question of how DAXX selectively targets misfolded clients over native proteins. It is hypothesized that other regions of DAXX contribute to modulate both its chaperone function and its selectivity [70]. These recent studies highlight that histone chaperones play integral and more direct roles in nuclear proteostasis beyond maintaining genetic integrity. Their mechanisms of action appear to be distinct from the canonical ATP-dependent chaperones. This additional nuclear chaperone capacity may reflect and buffer the metastability of the nuclear proteome in non-stress condition. Recent studies related to histone chaperones clearly outline the need to focus on their roles in maintaining nuclear proteostasis, MLOs, and their regulations upon misfolding stress. Especially noteworthy is the fact that histone chaperones, akin to histones, undergo extensive protein modifications, presenting fascinating opportunities for regulatory interactions with both their clients and nuclear biocondensates.

3.2.3. Other types of nuclear chaperones

A holdase activity has been identified for several nuclear proteins. This is the case of a range of transporters including Transportin-1, -2, and -3 but also the importins β and 7 [71–74]. These proteins have been shown to promote the transfer of the protein Fused in sarcoma (FUS) into the nucleus and to delay its aggregation. FUS is an RNA-binding protein with important roles in DNA and RNA processing [75]. Cytoplasmic accumulation of FUS is associated with neurodegenerative disorders. Similar to DAXX, these transporters recognize the basic NLS to translocate FUS into the nucleus. Upon binding to their cargos, these transporters also act as a holdase, delaying aggregation of other disease-related aggregation-prone proteins [73]. Interestingly, arginine methylation within the NLS decreases the stability of the complex, the efficiency of the import, and increases FUS aggregation. This is highly relevant for therapeutic intervention [72]. It is interesting to speculate, that other nuclear proteins with such amino acid biases, including ribosomal proteins, are chaperoned in a similar manner until their final destination, thereby avoiding non-productive interactions.

The TRIPartite Motif (TRIMs) proteins represent a distinct family in metazoans and is an example of PN coordination [76]. Indeed, some members of this large protein family of E3 ligases also display ATP-independent chaperone properties. TRIMs are characterized by an N-terminal TRIM/RBCC motif comprising a RING domain, one or two B-Boxes, and a coiled-coil region [77]. Recently, TRIM19 has been involved in the degradation of misfolded and aberrant proteins including pathogenic ones [78]. TRIM11 also displays a chaperone-like function but is localized in both cytosol and

nucleus. TRIM11 and TRIM19 were both reported to display ATP-independent chaperone function including disaggregation [79]. However, the molecular mechanism of these chaperones remains to be investigated.

3.3. Recruitment of cytoplasmic-resident chaperones in the nucleus upon proteotoxic stress

3.3.1. Recruitment of cytosolic HSPs

Upon proteotoxic stresses, cytoplasmic molecular chaperones translocate and accumulate into the nucleus. This has been discovered for all main chaperone families including sHSPs, HSP70s, HSP90s, HSP110s/BAGs and the co-chaperones HSP40 and HOP [13,15,80–87]. Most of these chaperones are larger than the 35 kDa size-limit of the NPC and cannot passively enter the nucleus. To enter the nucleus, some of them take advantage of a NLS allowing active nuclear import through importin-mediated transport. Additionally, HSP70 (HSPA1A/HSPA1B/HSPA8 in human) and HSP90 may enter the nucleus by binding to other nuclear imported proteins. However, in condition of proteotoxic stress such as hyperthermia or oxidative stress, conventional RanGTP-mediated nuclear transports are attenuated, raising questions about how chaperones specifically accumulate within the nuclear compartment in response to misfolding stress. This phenomenon presumably shields the nucleus from cytoplasmic misfolded proteins [43,88,89]. However, HSP70 is efficiently imported into the nucleus during proteotoxic insult [13–15,19,43,90]. HSP70 import is specifically mediated by the protein Hikeshi, which does not belong to the importin family [91]. Hikeshi is so far the only known nuclear importer of HSP70 and this has been leveraged to comprehend the function of nuclear HSP70 in control and stress condition. The lack of nuclear HSP70 activates the heat shock response under normal conditions and impairs the recovery of the cell from proteotoxic insults [44,45,92]. These phenotypes are abolished by expressing a nuclear targeted variant of HSP70 [91,92]. These results indicate that nuclear HSP70s are crucial to repair the proteotoxic damages, aligning with recent findings highlighting the contribution of HSP70 activity in conformational repair of nuclear proteins when the stress subsides [19–21].

3.3.2. Trafficking and roles of cytoplasmic chaperones in the nucleus

Following nuclear translocations, some chaperones and co-chaperones accumulate into the nucleolus, as observed with HSP70s. This pioneer observation was made nearly 50 years ago [13,15]. For a long time, the significance of such massive translocation and accumulation in the nucleolus remained elusive. It was initially postulated that HSP70 protects nuclear functions during stress, especially in ribosome biogenesis due to the aggregation propensity of ribosome proteins. Moreover, HSP70 activity is required to recover the normal morphology of the nucleolus when the stress subsides [14,19–21]. Recent findings reveal that, upon misfolding, a subset of nuclear proteins accumulates within the granular component (GC) phase of the nucleolus. HSP70 activity is required for the refolding of these proteins concomitantly to their exit from the nucleolus [19–21]. Ribosome proteins also enter this pathway and together with DNAJB6, HSP70 efficiently resolubilizes these proteins and ribosome biogenesis restarts after the stress [19,93]. Similarly, nucleolar storage preserves epigenetic marks upon proteotoxic stresses in an HSP70 activity dependent manner [21]. However, the determinants of HSP70 and HSP70 substrates nucleolar translocation are unclear. In a pioneering study, it was highlighted that a deletion of the C-terminal substrate-binding domain, with no chaperone activity, prevented both self-translocation and misfolded substrate translocation into the nucleolus upon stress. This suggests that HSP70 chaperone activity

is required for its nucleolar accumulation upon proteotoxic stress, concomitantly with client nucleolar translocation. Whether this effect is direct or indirect remains to be elucidated. HSP70 may maintain misfolded proteins soluble for other factors that could mediate their translocation within the nucleolus. Recent studies indicate that HSP70 and some substrates can be immobilized by ribosomal intergenic spacer noncoding RNA [94]. Additionally, HSP70 may recognize substrates within the nucleolus, such as orphaned unassembled ribosomal proteins [93]. Notably, HSP70 inhibition does not impair its stress-induced nucleolar accumulation suggesting that its activity is dispensable for its nucleolar translocation [21].

In some cells, HSP70 does not accumulate into the nucleolus but rather in or at the vicinity of nuclear speckles along with the disaggregation machinery [81,82,95,96]. Moreover, it has been shown that HSP70 together with DNAJB1 translocates misfolded proteins from the cytoplasm to the nucleus for proteasome-mediated degradation [19,97]. In this condition, inhibition of the proteasome activity promoted the accumulation of the client proteins into the nucleolus acting as an overflow compartment. However, the functional significance of this pathway remains to be investigated in particular for metazoans. It was suggested to be related to the high amount of active proteasomes within the nucleus. Nonetheless, translocation of aberrant proteins in the nucleus creates a significant risk for nuclear proteostasis. Thus, this pathway might represent a failsafe mechanism or an indirect effect occurring when the cytoplasmic PQC machinery is overwhelmed. The complex network, roles, and trafficking of cytoplasmic HSPs within the nucleus upon stress, especially considering the multitude of nuclear MLOs, remain to be elucidated.

4. Degradation of aberrant proteins in the nucleus

Regulated degradation of proteins is an essential mechanism to adjust the copy number of proteins and regulates virtually all processes and allows cells to adapt to changing conditions [98]. Misfolded or otherwise aberrant proteins are primarily degraded by the Ubiquitin-Proteasome System (UPS) preventing their accumulation and consequently precludes protein aggregation (Table 1) [99,100]. The nucleus of proliferating mammalian cells is rich in active proteasomes [101]. However, the UPS is also involved in targeted proteolysis of most proteins beyond damaged proteins and as such regulated most essential processes including cell cycle, transcription, and DNA maintenance [102]. Nuclear-targeted protein degradation is essential to maintain nuclear proteostasis. Indeed, accumulation of proteins targeted for degradation leads to proteotoxic stress, and these non-degraded proteins accumulate within the nucleus. Conversely, proteasome inhibition induces proteotoxic stress. Moreover, proteasome impairment is a toxicity mechanism induced by various cytoplasmic and nuclear aggregation-prone proteins related to conformational disorders [2].

4.1. Sources of nuclear misfolded proteins

The sources of nuclear misfolded proteins are highly diverse, arising from proteotoxic stresses such as heat stress or oxidative stress, and is also related to the rich metastability of the nuclear proteome [57]. Moreover, a number of disease-related aggregation-prone proteins accumulate in the nucleus and are degraded in this compartment such as poly-glutamine expanded mutant Ataxin-1 [78,103]. Additionally, over the last decade a wealth of evidence has accrued highlighting that aberrant translation products accumulate in the nuclear compartments. These Defective Ribosomal Products or DRiPs include aberrant translation termination, amino acids misincorporation, faulty or truncated mRNAs, and some other

non-conventional translation products are a major source of aberrant polypeptides [104]. Such products are normally cleared in a pathway involving cotranslational binding of both vasolin-containing protein (VCP) and HSP70 to the DRiPs and assist their ubiquitylation before degradation. Similarly, incompletely synthesized proteins from stalled mRNA translation are cleared on the ribosome by a pathway involving the Listerin E3 ubiquitin protein ligase 1 (LTN1) [105]. If they are not degraded efficiently, all these faulty ribosome products accumulate in cytoplasmic condensates such as stress granules but also in nucleoli and PML bodies, impacting their functions [20,106]. However, how they are degraded within the nucleus is unknown. In addition, it has been demonstrated that some cytoplasmic misfolded proteins are imported to the nucleus in an HSP70 and DNAJB1 dependent manner (see part 3.3.2) [97].

Numerous nuclear proteins are prone to degradation as a result of damage or stoichiometric imbalance, such as oxidatively damaged histones, which are subsequently cleared by the proteasome [107]. Similarly, orphan ribosomal proteins in stoichiometric excess in the nucleus are also degraded in a pathway involving the E3 ligases HUWE1 and UBE2O [108–110]. Reflecting the importance of clearing orphaned unassembled proteins, stoichiometric imbalance and accumulation of such proteins lead to human diseases known as ribosomopathies [111]. Additionally, aberrant proteins from the nuclear envelope need to be degraded. Indeed, mutations in nuclear membrane proteins are responsible of various human diseases [112]. Recently, one clearance pathway for such proteins has been shown to involve the nuclear membrane-resident E3 ligases RNF5 and HRD1 as well as the protein TMEM33. Moreover, the ubiquitin-conjugating enzymes E2 Ube2G2 and Ube2D3 are required [113]. Thus, the nucleus faces a variety of aberrant conformers to be degraded and not all substrates seem to accumulate at one nuclear location. In contrast to yeast where a specific UPS machinery is in place to maintain nuclear proteostasis, metazoan nuclear degradation of misfolded proteins remains far less understood and no clear picture has emerged yet making this field a fertile ground for future work.

4.2. Nuclear degradation machineries

In general, misfolded species are poly-ubiquitylated after recognition by the well-known ATP-dependent mechanism involving the successive cascade of E1, E2, and E3 enzymes [114]. The targeted protein is covalently linked to ubiquitin using the side chain of internal lysines or more rarely on the N-terminal amine [99]. The last acting enzyme, the E3 ligases, provide the specificity of the pathway in recognizing various degradation motifs (or degrons). The UPS represents more than 850 individual components in humans, the vast majority of them are E3 ligases and are found in various subcellular compartments. However, not all of them have a role in clearance of misfolded species. Poly-ubiquitylated substrates are targeted and recognized by the 26S proteasome, a large proteolytic machinery [115]. The catalytic activity of the proteasome resides within the central chamber of the 20S subunit, which is only accessible to unfolded polypeptides. Thus, targeted proteins are unfolded in an ATP-dependent process by the 19S regulatory particle that caps the 20S core subunit [116]. Targeting to the UPS can be reversed by the activity of the ubiquitin hydrolases (deubiquitylase) giving more opportunity for the refolding machinery to recover the native structure [117]. Recently, it has been shown that nuclear proteasomes can use Midnolin to recruit proteins targeted for degradation in an ubiquitin-independent manner [118]. This novel mechanism targets transcriptional regulators involved in the response to growth factors, neuronal, and immune stimuli. Mechanistically, the recognition

and selectivity involve the catch domain of Midnolin that binds onto degenerated amphipathic regions with the potential to form beta strand. Whether such Midnolin-degron is more widely used requires further investigations. How the Midnolin-proteasome pathway is regulated in various conditions to control transcriptional programs or other nuclear functions also requires further attention.

The efficacy of the UPS system also relies on additional factors. The protein p62/SQSTM1 is such a factor that is known to sequester ubiquitylated proteins in biocondensates and to shuttle them to autophagic degradation but also delivers ubiquitylated substrates to the proteasome through its PB1 domain [119]. While its function within the cytoplasm has been under scrutiny for a long time, its nuclear functions are emerging. p62 is able to shuttle between the cytoplasm and the nucleus through its two NLSs and one Nuclear Export Sequence (NES). In the nucleus, p62 was reported to have a recruitment role for both the UPS machinery as well as substrates including upon proteotoxic stress [120].

Recently, it has been shown in the budding yeast that the Intranuclear Quality Control (INQ) compartment that stores nuclear misfolded proteins can be degraded by the vacuole and involves the egress of the INQ from the nucleus [121]. However, such a mechanism has not yet been reported in metazoan cells. This potentially signifies an additional pathway for clearing aberrant proteins in organisms where mitosis is enclosed, preventing the dispersion of nuclear misfolded species into the cytoplasm during mitosis. Further investigations are needed to explore the applicability and mechanisms of this clearance route in metazoan cells, providing valuable insights into nuclear proteostasis.

4.3. Nuclear degradation and chaperone interplays

Molecular chaperones play key roles in targeted nuclear protein degradation of misfolded proteins. The proteasome is unable to process aggregated proteins. Thus, prior degradation polypeptides need to be extracted from the aggregate in a process called disaggregation. Moreover, canonical and non-canonical chaperones, by their holdase functions, preserve misfolded proteins in a non-aggregated state thereby keeping them competent for either refolding or proteasome degradation. The chaperone system works together with the E3 ligases for the recognition of damaged proteins. This is the case for example with the E3 ligase CHIP (C-terminal HSP70 Interacting Protein) which interacts with the carboxy terminus of HSP70 and HSP90 via its tetratricopeptide repeat (TPR) domain to add ubiquitin on chaperone-bound substrates [122–124]. Interestingly, upon non-productive cycles of (re) folding characterized by a prolonged chaperone residence time, the misfolded client may be triaged for degradation through ubiquitylation. CHIP also displays a holdase capacity. CHIP is enriched in nucleus and translocates into the nucleolus upon proteotoxic stress along with misfolded proteins [19,125]. CHIP seems to be part of the machinery involved in the management of nucleolar misfolded proteins [125]. However, its precise role in nuclear PQC remains to be discovered. The metazoan E2/E3 enzyme UBE2O also displays a holdase function and ubiquitylates unassembled “orphan” proteins and misfolded proteins for quality control [108,126]. Interestingly, UBE2O utilizes the histone chaperone NAP1L1 to selectively recognize a subset of clients. Thus, UBE2O mediates ubiquitylation without a separate E3 ligase [108,126].

The ATPase VCP has crucial functions in a variety of important cellular processes including PQC as it serves as a molecular engine for unfolding polypeptide chains [127]. VCP has also known roles in the nucleus including maintaining genomic integrity [128,129]. Mutations within VCP are linked to neuromuscular diseases and cancer [130]. VCP interacts with numerous co-factors mainly for

selectivity. The VCP nuclear Cofactor Family member 1 and 2 (VCF1/2) bind to VCP directly via an alpha-helical motif and associate to other factors [129]. VCF1/2 localize to the nucleus and promote the nuclear import of VCP. However, the nuclear functions of VCP remain to be investigated in particular upon proteotoxic stress. A role in ribosome biogenesis has been postulated [131]. Nuclear VCP may disaggregate oligomers and maintain soluble misfolded proteins during proteotoxic stress to allow further processing such as trafficking, degradation, or refolding. Clearly, future work will identify nuclear PQC roles of VCP.

4.4. Multi-tagging in degradation of nuclear misfolded proteins

Ubiquitin-like molecules are a family of protein modifiers. Among them, the Small Ubiquitin-like Modifier (SUMO) 1,2,3, and 4 are particularly relevant for nuclear functions and proteostasis. SUMOs are slightly larger than ubiquitin (~11 kDa) and can be conjugated to target proteins as monomers or various types of polymers. SUMO is attached to its target via the classic E1-E2-E3 enzymatic cascade similar to ubiquitin. Strikingly, SUMO targets predominantly nuclear proteins and regulates almost all nuclear processes. Sumoylation is an essential and dynamic post-translational modification. Sumoylated proteins are recognized by protein domains known as SUMO Interaction Motifs (SIMs). In particular, recent developments of the field of LLPS biology have pinpointed the role of sumoylation in nuclear body assembly [132]. Moreover, dysregulation of sumoylation is linked to diseases including cancer, highlighting the crucial role of sumoylation in physiology. For a complete review on SUMO and its impact we suggest the following review [133].

SUMOs have identified roles in clearing nuclear aberrant proteins. It has been shown that sumoylated aberrant proteins are ubiquitylated before proteasome-mediated degradation [78]. Thus, SUMO in this case serves as an upstream marker allowing the recognition of misfolded proteins by the ubiquitylation machinery. SUMO polymers are recognized by SUMO-Targeted Ubiquitin Ligases (STUBL) that ubiquitylate the target protein and promote its degradation [133]. Mechanistically, STUBLs recognize SUMO polymers via their multiple SIMs [134,135]. SUMO-targeted ubiquitin proteases (STUbPs) exert the opposing activity. In the nucleus, it has been reported that PML/TRIM19 is able to sumoylate misfolded nuclear proteins including the pathogenic poly-glutamine expanded ataxin-1 and huntingtin exon 1, the aggregation-prone protein TDP-43, and a nuclear misfolded protein model. Then, the STUBL Ring Finger protein 4 (RNF4) mediates the ubiquitylation of these proteins, facilitating thereby the degradation of faulty proteins [78]. The cellular significance of this additional tagging remains to be elucidated. Because of the involvement of sumoylation in biocondensates, this modification might assist the protein for its trafficking. Alternatively, the modification might simply help in maintaining the protein soluble, preventing thus its aggregation, and keeping it competent for UPS-mediated degradation.

5. The liquid-liquid phase separated compartments of nuclear protein quality control

The nucleus, teeming with dynamic Membrane-Less Organelles (MLOs, Fig. 2), presents a complex and sensitive environment that prompts an investigation into how PQC pathways are spatially orchestrated. For a long time, concentrated proteins at one location was only seen as an aggregated deposit, possibly linked to cellular pathologies. Indeed, it is known that misfolded proteins can collapse together in cells as insoluble inclusions and are linked to various diseases. However, over the last 20 years a wealth of evidence has accrued, highlighting that cells triage or deposit aberrant

proteins to specific cellular locations as a part of a strategy to manage them [16]. This concept was pioneered by the lab of J. Frydman who showed that misfolded proteins are deposited into distinct compartments depending on the ubiquitylation of the aberrant proteins. One cytosolic deposition site, with amyloid-like and arrested features, is called the Insoluble PrOtein Deposit (IPOD), and another one for ubiquitylated misfolded proteins was initially named JUxtaNuclear Quality control compartment (JUNQ) [18]. The JUNQ site was thought to be in the yeast cytoplasm but later its localization was confirmed to be within the nucleus next to the yeast nucleolus and renamed IntraNuclear Quality control site (INQ) [136]. In human cells, the cytoplasmic aggresome was suggested to be the equivalent of the JUNQ/INQ as this perinuclear structure also accumulates ubiquitylated aberrant proteins and PQC components [137]. Importantly, while the insoluble and inert IPOD seems to be a final destination for aberrant proteins, JUNQ/INQ appears to be only a transient deposition center for misfolded proteins before their degradation [18,136]. More generally, the reasoning behind such transient deposition sites is that they are maintaining aberrant proteins into a state competent for further refolding or degradation, or simply to protect cells from the toxicity of soluble species [17–19].

Perhaps the first biocondensates to consider in nuclear proteostasis are the nuclear Heat Shock Factor 1 (HSF1)-stress bodies or granules (Fig. 2). HSF1 is the master regulator of the heat stress response that rapidly and reversibly regulates a transcriptional reprogramming to fight proteome damages. HSF1 forms small nuclear biocondensates via LLPS at heat shock protein gene loci and also enable the concentration of the transcription machineries to promote the transcription of target genes [138]. Such phase separation capacity appears to be regulated by at least specific phosphorylation events on HSF1. However, prolonged stress promotes liquid to gel transition of HSF1-nuclear bodies with impaired reversibility [50,138]. After the stress, HSP70 disperses HSF1-stress bodies to attenuate the transcriptional response [138]. Thus, the activity and regulation of HSF1 is coordinated through LLPS processes allowing a dynamic and reversible transcriptional reprogramming upon adverse conditions.

5.1. Membrane-less organelles and protein aggregation

Essential nuclear functions depend on a number of liquid-like phase separated compartments. These compartments are non-stoichiometric assemblies of proteins and nucleic acids. They are membrane-less and their components contain interacting modules (valence) allowing them to be a part of the condensate. The type of interaction dictates the biophysical properties of the organelle. Due to these properties, MLOs are highly sensitive to environmental changes and can rapidly respond to changing cellular conditions, making them prevalent in the field of proteostasis. Indeed, many of them form rapidly upon proteotoxic stress. Additionally, some of these assemblies are also recognized and dispersed by molecular chaperones [22–24]. Thus, molecular chaperones are also able to regulate MLOs. They can also serve as overflow compartments for misfolded proteins highlighting their importance in the cellular sorting and fate of such proteins [19,21,28]. Interestingly, aggregation-prone proteins, including disease-related proteins, often have the capacity to form such condensates and/or to interact with other cellular MLOs and disturb their properties and functions [25,26,139]. Moreover, it has been reported that the crowded environment of biocondensates can both promotes and precludes protein aggregation [19,140,141]. In one hand, the crowded environment within biocondensates can promote the nucleation and liquid-to-solid transition of aggregation-prone proteins upon excess of homotypic interactions between the aggregation-prone

proteins. In the other hand, the network of heterotypic interactions within MLOs can functionally work as a holdase and prevents irreversible aggregation.

Over the last 15 years, such new description of the cellular interior has revolutionized our perception of the cellular content and how cellular biochemistry is organized. This field strongly intersects with the study of PQC and proteostasis in particular for the subcellular management of misfolded proteins in the nucleus. Indeed, there is accumulating evidence suggesting that biocondensates themselves can serve as PQC compartments where misfolded proteins selectively partition into biocondensates. In the case of the nucleus, it has been shown that misfolded proteins accumulate in nucleoli and PML bodies [19–21,78].

5.2. Sequestration in membrane-less organelles as a strategy to cope with misfolded proteins

Since the initial discovery multiple studies have demonstrated that misfolded proteins can be recognized and triaged into spatially restricted cellular locations as an integral strategy to cope with an increase in misfolded proteins and is particularly relevant for the nuclear compartment [16,18,19,21]. These deposits generally fulfil several PQC functions. While some of them have been characterized with great details, their exact functions and cellular significance are still being under active investigations. Intuitively, these deposits can represent a reaction crucible where misfolded proteins accumulate at the site of their elimination or processing where all required machineries are also concentrated [19,21,120]. This sequestration may also represents a transient storage providing the cell with a time window to restore proteostasis and thereby prevents proteostasis collapse [142,143]. Indeed, subcellular sequestration protects the PN from being overwhelmed. Furthermore, these deposition sites appear to also mitigate the toxicity of misfolded species [19]. Sequestered within a defined subcellular location, aberrant proteins will not or much less interact with the cellular milieu and thus off-pathway toxic interactions will be prevented. In particular, many of these deposition sites feature ATP-independent molecular chaperones that will in addition to shield misfolded proteins also maintain them in a state competent for further processing. Other functions have been postulated such as signaling but require further investigations. Thus, subcellular sequestration of misfolded proteins is an integral strategy to cope with proteotoxic stress.

5.3. Nuclear degradation sites

The selective degradation of misfolded proteins seems to involve often transient storage or sequestration in MLOs in particular upon proteotoxic stress. This may reflect that these compartments act as overflow compartments [19,20,120]. Additionally, there are evidences highlighting that accumulation within biocondensates delays degradation and provides the cell with more opportunity to refold or remobilize the protein initially targeted for degradation [144]. This delayed degradation appears to be relevant for unassembled ribosomal proteins because their synthesis account for a large fraction of total protein synthesis. Therefore, remobilizing ribosomal proteins saves considerable amount of energy [93]. Nonetheless, the UPS machinery and its substrates accumulate in nuclear compartments including PML bodies and nucleoli upon proteotoxic stress [19,21,78,145]. However, whether they fulfil only their degradative role is debated. Indeed, various studies report that ubiquitin and ubiquitin-like molecules could play extended roles including increased solubility of the modified proteins, handle for the ubiquitin-dependent remodeler VCP, and potentially a role in targeting them to the biocondensates [132,146].

Recently, a role of p62 in spatial nuclear PQC has been identified. In the nucleus, p62 is involved in the formation of phase separated bodies that recruit both the UPS machinery as well as its substrates [120]. Conceptually, concentrating all components of a pathway within a defined location has been proposed to be one the main function of cellular biocondensates. Such nuclear p62 foci were shown to be efficient at degrading substrates in control conditions but also upon proteotoxic stresses [120].

It was shown that various cancer-related proteins including p53 accumulate in the nucleolus or in a structure originating from it upon proteasome inhibition [145]. This was named “nucleolar aggresome” as it shares several similarities with its cytoplasmic counterpart including ubiquitylated proteasome substrates. Moreover, such structure is also rich in poly-A RNA. Interestingly, conjugation to ubiquitin drives the accumulation of these proteins to the nucleolar aggresome and deubiquitylation prevents substrate accumulation at nucleolar aggresomes. However, enlarging the cellular pool of ubiquitin caused the dissolution of the nucleolar aggresome implying an additional role of ubiquitin in the trafficking of the substrates to nucleolar aggresomes [145].

Perhaps the best-described clearance pathway for nuclear misfolded proteins involves the PML bodies. In contrast to other nuclear bodies, they are assembled in an RNA-independent manner and are enriched in the SUMO machinery. Indeed, sumoylation and SUMO-dependent interactions are essential events in forming liquid-like phase separated PML-bodies [132]. It has been reported that the protein PML/TRIM19 selectively recognizes misfolded proteins and acts as a SUMO E3 ligase by adding SUMO2/SUMO3 moieties on the protein substrates. SUMO2/3 chains are then recognized by the STUbL RNF4, which ubiquitylates sumoylated substrates and thus directs them to proteasome-mediated degradation (see also part 4.4) [78]. Various misfolded and aberrant proteins are substrate of this pathway and include misfolded protein models, pathogenic poly-glutamine expanded proteins, and DRiPs [20,78]. However, conflicting evidences exist for some misfolded proteins since they can be targeted to both PML bodies and nucleoli [19,21,78]. Possibly, this represents alternative pathways that can co-exist, or depending on the conditions or the existing machinery one pathway may become prevalent.

5.4. Cellular protection by nucleolar-originated bodies

Ultimately, spatial sequestration lowers to probability of aberrant off-pathway interactions promoted by the misfolded protein species. Perhaps the nucleolus, ribosome factory of the cell, represents best such effect. These last years a number of studies have demonstrated the positive role of the nucleolus (or a structure originating from it) in protecting nuclear homeostasis and functions.

Upon proteotoxic stresses, a vast number of nuclear proteins are modified by ubiquitin and ubiquitin-like molecules. Among the ubiquitin-like molecules, NEDD8 (NEural precursor cell expressed Developmentally Down-regulated 8) which is the closest homolog to ubiquitin has been spotted recently as a player safeguarding the nuclear proteome during proteotoxic stress. NEDD8, like ubiquitin, is conjugated to target proteins using the three-steps E1-E2-E3 mechanism using dedicated and specific enzymes [147]. However, upon proteotoxic stress such as heat stress, oxidative stress or proteasome inhibition an alternative neddylation machinery operates resulting in the production of substrates tagged with hybrid ubiquitin/NEDD8 chains. The production of such chains has been attributed to the E3 ligase HUWE1. Interestingly, this piece of data are related to the previously discovered role of HUWE1 in the degradation of orphaned ribosomal proteins in the nucleus [109,110]. As compared to the canonical ubiquitin chain, these

hybrids ubiquitin/NEDD8 chains do not allow for an efficient degradation of the modified substrate. In an elegant study, the biological significance of such tagging during proteotoxic insults was revealed [143]. Indeed, this specific protein modification promotes the aggregation of the target proteins in insoluble inclusions that are related to the nucleolus (termed nucleolus related inclusions). Indeed, during stress the ubiquitin/NEDD8-tagged substrates appear to phase separate from the nucleolar compartment and form a structure resembling a proteinaceous inclusion. These inclusions contain nucleolar and ribosomal proteins. It has been shown that this pathway protects the nuclear UPS from being overwhelmed by a sudden increase in protein substrates, as it is the case upon proteotoxic stresses. At the molecular level NEDD8 prevents canonical substrate ubiquitylation and therefore proteasomal degradation [143]. Such specific protein tagging may tune the biophysical properties and solubility of the modified proteins, driving them to forming another phase or inclusion. However, this remains to be demonstrated formally.

Other structures that seem to originate from the nucleolus have been described. This is the case of nucleolar deposits termed amyloid-bodies (A-bodies) that were observed upon heat stress or acidosis [94,148]. Interestingly, A-bodies display amyloidogenic properties but could be solubilized in a HSP70-dependant manner upon recovery from stress. A-bodies are seeded and immobilized by stress-induced ribosomal intergenic spacer noncoding RNA [94]. Moreover, this physiological amyloidogenesis appears to be related to cell adaptation to stressors [148,149]. Altogether, with the previously described nucleolar aggresome (see part 5.3) it is not clear how all these structures originating from the nucleolus are related to each other's. Nonetheless, there are extensive overlaps between the proteins identified in these compartments. They all seem to share the features of a transient storage system preventing the nuclear PN to be overwhelmed. They also all rely on PQC components to be resolubilized when the stress subsides.

5.5. The nucleolus as a protein quality control compartment

5.5.1. Nucleolar translocation of misfolded proteins

The nucleolus itself has been shown to accommodate a number of proteins when the cell is facing proteotoxic insult (Fig. 3B). The nucleolus is a prominent nuclear MLO with liquid-like properties, well known for its role in ribosome assembly among other functions [51]. Three main immiscible phases, the fibrillar center, dense fibrillar component, and GC, compartmentalize the nucleolus and correspond to various steps of ribosome assembly [51,150]. The GC, where pre-rRNA processing and assembly steps of pre-ribosomal particles occur, is rich in proteins with IDR and amino acid compositional bias such as NPM1 and have been shown to drive phase separation in vivo and in vitro [150–153]. Using protein reporters, it has been shown that misfolding is a pre-requisite for the translocation into the nucleolus (Fig. 3B) [19]. Within the nucleolus, misfolded proteins accumulate homogeneously within the GC of the organelle and become clients of this nucleolar phase [19,21]. The vast majority of proteins that accumulate in the nucleolus are nuclear proteins. Nonetheless, it has been shown that DRiPs can also accumulate in the nucleolus with attendant consequences on its functionality [20,106]. Moreover, a number of disease-related proteins also invades the nucleolus with negative effects on its properties and functions [19,25,26,28,139]. This is the case of dipeptide repeat proteins, produced in the course of C9orf72-linked amyotrophic lateral sclerosis and frontotemporal dementia, that alter the biophysical properties of the organelle [19,25,26]. Ribosome proteins also enter this pathway upon proteotoxic stress [19,21]. It has been found recently that unassembled or orphaned ribosomal proteins during proteotoxic stress are stored in the nucleolus

allowing to resume rapidly ribosome and protein biogenesis after the stress [93]. Other proteins translocate into the nucleolus upon stress including crucial epigenetic regulators [19,21]. For example, the subunit Polycomb repressive complexes (PRC) 1 and PRC2 as well as various chromatin modifiers reversibly translocate into the nucleolus upon heat stress. Here also, nucleolar storage allows to re-establish the epigenetic landscape when the stress subsides providing a biological significance to such pathway [21].

Along with these misfolded proteins, a large number of known PQC effectors accumulates in the GC of the nucleolus (Fig. 3B – Table 1). This is exemplified by HSP70 as it has been observed in the pioneer studies [13,15,90]. How HSP70 translocates in the nucleolus is still a matter of debates [90,94]. HSP70 could recognize nucleolar resident components, or misfolded proteins. Additionally, HSP70 may enter the nucleolus alone or in complex with a small chaperone and/or a misfolded substrate. These exciting questions need further investigations. Nevertheless, the activity of HSP70 has been linked to the refolding of the nucleolar misfolded proteins, their repartitioning outside the nucleolus, and the efficient recovery of their functionality [19,21,93]. All these data explain the observations made 40 years ago where HSP70 was required to recover the shape of the nucleolus after stress [14]. The functions of the other identified PQC effectors in the nucleolus upon stress remain to be investigated.

5.5.2. The liquid-like properties of the nucleolus enable its PQC functions

Interestingly, the biophysical properties of the nucleolus appear to be essential for the functionality of this pathway (Fig. 3C). It has been shown that altering the liquid-like properties of the nucleolus impaired the reversibility of this process and resulted in amyloidogenic transformation of the proteins [19]. Indeed, dissolving the GC phase resulted in irreversible protein aggregations upon proteotoxic stresses. This indicates that the GC phase of the nucleolus maintained misfolded proteins in a state competent for further refolding by the HSP70 machinery. At the molecular level, it is likely that misfolded proteins expose domains or protein regions that will engage in multivalent interactions with nucleolar protein such as NPM1 and nucleolin and thus become clients of the GC phase. This network of heterotypic interactions within the GC provides a holdase function by buffering the homotypic interactions between the aberrant proteins (Fig. 3C). Indeed, the GC phase of the nucleolus is able to prevent the co-aggregation of two different aberrant proteins highlighting the strength of its holdase function [19]. Additionally, we showed that increasing the burden of the GC with more misfolded proteins impaired the reversibility of the pathway and triggered amyloidogenic transformation [19]. Similarly, affecting the GC phase of the nucleolus with disease-related proteins impaired the reversibility of this process (Fig. 3C) [19,28]. Upon excess of misfolded proteins, the holdase capacity of the GC is exceeded which results in homotypic interactions between misfolded proteins and thus irreversible aggregation (Fig. 3C) [19,28]. Excess of homotypic interactions is known to promote biocondensate ageing or pathological liquid-to-solid transition [53].

Moreover, results showed that the interactions between misfolded proteins and nucleolar proteins are more stable than interactions between nucleolar resident proteins themselves [20,21,28]. As such, misfolded proteins arrested a fraction of nucleolar proteins and thus entered in competition with ribosome particle maturation contributing to halt ribosome biogenesis (Fig. 3C). Finally, the nucleolar environment prevented the toxicity of amyloid forming proteins [19]. Therefore, the holdase capacity of the nucleolus allows for various PQC functions including keeping proteins in a refolding-competent state, efficient and cost-effective recovery of various essential nuclear functions following the stress,

preserves the nuclear PN upon stress, and protects cells from toxic proteins. Future work will need to focus on both regulators of these nuclear biocondensate-associated PQC mechanisms and to understand how they are affected during aging and pathological conditions.

5.6. Fate of stored misfolded proteins: refolding or degradation?

The determinants of the decision between refolding and degradation for misfolded proteins within nuclear bodies is for the large part unknown. As example, within the nucleolus the machineries for both degradation and refolding have been identified [19–21]. The nexus of such activities is potentially the E3 ligase CHIP that cooperates with both the degradation and refolding branches of the PN (see § 4.3) [125]. Most likely, a key parameter is the concentration of the different PQC effectors on site relative to the concentration of misfolded proteins and the efficiency of the refolding machinery. However, some biocondensates are more specialized. It seems to be the case for p62 biocondensates where the refolding machinery has not been identified while the whole UPS machinery is in place [120]. Nonetheless, it is clear that all these bodies are here to provide a sufficient time window for the cell to deal with the amount of misfolded proteins in order to avoid irreversible damages. Given the energy cost of producing some of the proteins targeted by these pathways, which can account for up to half of the produced proteins, it is reasonable to consider that cells try to refold such proteins rather than to destroy them. Recent works focusing on ribosome proteins appear to point towards that direction [93]. Protein modifications may play a key role in this decision since they can affect material properties and recognition of the misfolded proteins. Indeed, upon proteotoxic stress it was reported that the ubiquitin pool is limiting and stress-induced neddylation promotes aggregation and prevents degradation [143,145]. Nonetheless, future work will need to concentrate on the recovery events and factors, which are so far poorly investigated.

5.7. Coordination and communication between biocondensates in nuclear proteostasis and in quality control

Biocondensates have a specific set of core molecules often referred as “scaffold” components and thus provide unique molecular environments. Therefore, biocondensates with different compositions do not interact, combine, fuse or coalesce. This is exemplified by multiphasic biocondensates where various liquid phases co-exist without mixing. Recent studies propose a role of the network of protein-RNA interaction in the multiphasic organization of biocondensates [154]. The nucleolus is such a complex, multilayer biocondensate with different co-existing densities arising from a combination of active processes as well as spontaneous phase transitions driving complex coacervation [155]. Moreover, the network of nuclear actin and the viscoelastic properties of the chromatin preclude long-range movements of large biocondensates [150].

Nonetheless, the diversity and amount of nuclear biocondensates with PQC functions question whether inter-biocondensates coordination exists and contributes to cope with misfolding stress. As far as we know, there has not been any evidence of communication between biocondensates in nuclear PQC thus far. It has been reported that some proteins are able to accumulate in various biocondensates upon stress [19,81,82,90]. This is the case of HSP70 that accumulates at nuclear speckles and nucleoli upon heat stress but also dissolves A-bodies and HSF1-stress bodies [81,94,138]. Moreover, the protein PML/TRIM19 associates with the nucleolus for p53 proteostasis (for review see Ref. [156]). Hence, the possibility of communication between biocondensates in nuclear

protein quality control (PQC) awaits formal identification and thorough investigation.

5.8. RNAs: novel players in nuclear proteostasis and properties of membrane-less organelles

The nucleus, abundant in RNAs, is proposed to have pivotal involvement in maintaining nuclear proteostasis and mediating membrane-less organelles (MLOs). Indeed, the RNA quantity within the nucleus can prevent aggregation of disease-related proteins [157]. Nevertheless, in contrast to the extensively studied roles of proteins within biocondensates, the functions and impacts of RNAs have received considerably less scrutiny. However, mounting evidence suggests their involvement in shaping the properties of biocondensates, particularly under stress conditions.

The nucleolar aggresomes were described containing poly-A RNAs, which could possibly be mRNAs or lncRNAs [145]. However, they do not contain precursors or rRNAs. lncRNAs, which are derived from intergenic spacer region of the rDNA cassette, were identified in A-bodies. These lncRNAs are of low complexity and are thought to have no secondary structure [94]. Such extended conformation potentially provides a high valency for the recruitment of multiple partners bringing them into close proximity and trigger condensation. Various studies on phase separation of RNA-binding proteins have highlighted the critical role of RNAs in their condensation [158]. Furthermore, RNA has dramatic effects on the phase behavior of pathological biocondensates on both their formation as well as material properties. This was discovered for polyglutamine expanded pathological protein variants [159,160]. Interestingly, upon inhibition of the RNA polymerase I, which transcribes rRNAs, the nucleolus disassembles and misfolded proteins that initially accumulated within the GC phase of the nucleolus (containing both mature and precursors rRNAs) form irreversible aggregates with pathogenic features [19]. Albeit speculative, this suggests a contribution of rRNAs in buffering misfolded proteins in this compartment. Altogether, this is in line with recent studies focusing on the role of RNAs in biocondensate formation. Indeed, RNA in protein-free solution can undergo phase separation and percolation. Percolation is the formation of a system-wide spanning network of crosslinks. Indeed, within complex biocondensates such as the nucleolus, RNAs and RNA production can lead to viscoelastic properties with complex rheology extending the range a properties beyond simple liquids or gels [155]. Therefore, RNAs may contribute to dynamically arrest protein partners but also potentially prevent or delay pathogenic phase transition. The diverse functions of RNA underscore the importance of investigating their roles in nuclear PQC more extensively. It is crucial to recognize RNAs as integral components of biocondensates, akin to the protein fraction.

5.9. Various stressors, various outcomes

The variety of bodies and pathways identified so far may be related to the conditions and specific stressors used to characterize them. In particular, biocondensates are highly sensitive to environmental changes. It is also clear that these pathways seem to require a precise equilibrium of effectors at the crossroad between refolding, degradation, and trafficking. It is thus of prime importance to consider the stressor used and its primary targets. It is likely that proteins will respond differently to the each stressor. In particular, while some stressors directly inhibit parts of the PN, some others directly challenge protein structure. For example, inhibiting the proteasome is a widely used stressor that primarily results in the accumulation of proteins targeted for proteasome-mediated degradation. Because impacted proteins cannot be

degraded, proteasome inhibition is a potent stressor to study stress-induced biocondensates. Differently, heat stress directly challenges protein fold thus it primarily affects the metastable proteome. In such condition, metastable proteins misfold (at least partially) which creates a sudden burden for the PN. Some of these proteins will be degraded by the proteasome but its capacity is limited. Thus, many misfolded proteins will escape degradation and will accumulate in stress-related bodies. Inhibiting the proteasome activity prior to heat stress often worsen the heat stress phenotype suggesting that some of these bodies serve as overflow compartments [19,20]. The extent or strength of the stressor also need to be considered as it is directly correlated to the amount of misfolded proteins produced. For the nucleolus, excess of misfolded proteins correlates with amyloidogenic transformation [19]. In general, prolonging stress will generate more misfolded proteins, increase the burden of the PN, promote irreversible transformations, and compromise more functions. Interestingly, as highly proliferating cells possess larger and more nucleoli they might be able to cope better with nuclear misfolded proteins and may represent therapeutic targets.

6. Conclusion and future directions

The past decade has witnessed the rapid evolution of the field of MLOs. Concomitantly, research on compartment-specific PN has also developed. Given the nucleus's abundance of Membrane-Less Organelles (MLOs), the question of where aberrant nuclear proteins are handled has emerged. Moreover, how the different branches and activities of the nuclear PN interact with each other's in time and space remains elusive despite some initial studies. While protein biosynthesis does not occur (or minimally) in this compartment, the nucleus is exposed to misfolded proteins upon proteotoxic stresses but also upon loss of nuclear envelop integrity or nuclear pore complex selectivity as is the case during aging. Strikingly, the nuclear PN appears adapted to its proteome with a collection of ATP-independent chaperones that maintains the solubility of essential nuclear proteins. These ATP-independent chaperones have been identified in the past as histone chaperones but it becomes apparent that they play a broader role in the nucleus in particular upon misfolding stress (Fig. 3C). They display unique features including how they recognize their substrates. Such chaperones primarily recognize and engage in electrostatic interactions much more likely than in exposed hydrophobic regions. Furthermore, they work in the dilute phase as well as in nuclear biocondensates forming a network of heterotypic interactions buffering the pathological homotypic interactions of aberrant proteins and thus providing a novel holdase capability. Nuclear biocondensates, in particular PML bodies and nucleoli, play central roles in nuclear PQC as they accumulate both misfolded protein substrates and PQC components allowing efficient processing and recovery when the stress subsides.

Misfolded proteins within the nucleolus are refolded by the HSP70 system allowing their repartitioning to the nucleoplasm. However, whether the PQC system has a role on biocondensates themselves (scaffold proteins) in these conditions beyond the misfolded proteins is unknown. Nonetheless, the nuclear PQC machinery is emerging as a regulator of biocondensate formation, dissolution, and biophysical properties. Altogether, the last 20 years of research has clearly shed light on the specificity of the nuclear PQC apparatus, positioned nuclear biocondensates as PQC nexus as well as opened new avenues for exciting discoveries. Hereafter we compiled outstanding questions that need further investigations.

- For most of the described pathways, little to no regulations have yet been discovered. We expect important and various levels of

regulation controlling nuclear proteostasis. In particular protein modifications are suspected to play key roles as they can tune biocondensate formation and biophysical properties. Protein modifications can also modify the properties, activity or availability of condensate-forming proteins.

- Studies have uncovered that misfolded or otherwise aberrant proteins can accumulate into various nuclear biocondensates. However, the determinants of such triage is unknown and discrepancies exist in the literature. In particular, the role of HSP70 in the nuclear sorting of aberrant proteins have been suggested long time ago but is not clearly demonstrated.
- How these pathways are affected in diseases and during aging remain to be addressed at the mechanistic level. In particular, the nucleolar compartment has also been associated with aging and is a target of various disease-related proteins.
- The list of human diseases with nuclear aggregation-prone proteins or nuclear aggregates increases. However, the molecular pathology and toxicity mechanisms are for most of them poorly understood. This is partly because of the simultaneous presence of cytoplasmic aggregates masking or blurring the contribution of nuclear aggregates to the toxicity. Understanding nuclear specific toxicity pathways hold great promises for new therapeutic strategies.
- It becomes of prime importance to have a holistic consideration of nuclear proteostasis. In particular, the crosstalk between nuclear PQC and DNA/RNA quality control remains largely unexplored as well as potential coordination between biocondensates. It is expected that failure to maintain nuclear proteostasis will negatively impact genomic integrity and fidelity of the genomic information with attendant consequences on daughter cells feeding a vicious circle of increasing production of aberrant proteins over generations.

Addressing these questions will not only advance our knowledge of the circuitry operating upon proteotoxic stress and pathological conditions but will also shed light on pathways at play in physiological conditions. Moreover, studying nuclear PQC pathways in cells and tissues that are more closely related to the disease than rapidly growing cells will likely provide novel information.

Disclosure and competing interest statement

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Julia Buggiani: Writing – original draft, Writing – review & editing. **Thierry Meinel:** Funding acquisition, Writing – original draft, Writing – review & editing. **Carmela Giglione:** Funding acquisition, Writing – original draft, Writing – review & editing. **Frédéric Frottin:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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