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Autophagy as a caretaker of nuclear integrity

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Due to their essential functions, dysregulation of nuclear pore complexes (NPCs) is strongly associated with numerous human diseases, including neurodegeneration and cancer. On a cellular level, longevity of scaffold nucleoporins in postmitotic cells of both *C. elegans* and mammals renders them vulnerable to age-related damage, which is associated with an increase in pore leakiness and accumulation of intranuclear aggregates in rat brain cells. Thus, understanding the mechanisms which underpin the homeostasis of this complex, as well as other nuclear proteins, is essential. In this review, autophagy-mediated degradation pathways governing nuclear components in yeast will be discussed, with a particular focus on NPCs. Furthermore, the various nuclear degradation mechanisms identified thus far in diverse eukaryotes will also be highlighted.

Keywords: Atg39; NPC-phagy; nuclear blebbing; nuclear lamina; nuclear pore complex; nuclear-derived vesicle; nucleophagy; Nup159; quality control; selective autophagy

Autophagy describes a regulated process by which cellular material is imported into a degradative compartment, such as the vacuole or lysosome, for destruction. Three main types of autophagic processes have been described and extensively reviewed in detail elsewhere: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy [1–3]. In microautophagy or CMA, cargo is delivered directly to a degradative compartment, accomplished either through invaginations or protrusions of the lysosomal or vacuolar membrane or by translocation through a protein-mediated channel, respectively. In contrast, macroautophagy describes the process of cargo sequestration into a *de novo* synthesized double-membrane vesicle, called the autophagosome, which subsequently fuses with the lysosome/vacuole [4,5]. During autophagosome biogenesis, transfer of lipids from various intracellular membrane sources facilitates the expansion of an initial flat membrane disk to form a closed autophagosome via a cup-shaped intermediate, termed the phagophore or isolation membrane [6]. Importantly, macroautophagy and the protein machinery required for autophagosome formation are highly conserved from yeast, where it was originally characterized, to higher eukaryotes such as plants and mammals [7]. Interestingly, many of the core autophagy machinery proteins required for macroautophagy also play essential roles in microautophagy [3]. Together, autophagic processes ensure the specific targeting and removal of a wide range of damaged or unwanted cellular components, such as organelles, protein aggregates, and intracellular pathogens, thereby maintaining cellular integrity and homeostasis.

The nucleus is the defining feature of eukaryotic cells and is the primary location for organization and

Abbreviations
AGS, Aicardi–Goutières syndrome; AIM, Atg8 interacting motif; ASS1, argininosuccinate synthetase; CCF, cytoplasmic chromatin fragment; CMA, chaperone-mediated autophagy; ER, endoplasmic reticulum; Hd1, Heading date 1; IDR, intrinsically disordered region; INM, inner nuclear membrane; LINC, linker of nucleus and cytoskeleton; LIR, LC3-interacting region; MAP1LC3B/LC3B, microtubule-associated protein 1 light chain 3 beta; MEF, mouse embryonic fibroblast; NDV, nuclear-derived vesicle; NPC, nuclear pore complex; ONM, outer nuclear membrane; rDNA, ribosomal DNA.

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expression of the genome (Fig. 1A). In addition to DNA and several classes of RNAs, the nucleus houses a distinct proteome of architectural proteins and gene expression machinery. Many nuclear proteins are further organized into specialized subdomains, such as the nucleolus, enabling the compartmentalization of nuclear functions [8]. Nuclear content is separated from the surrounding cytoplasm by the nuclear envelope, which is continuous with the endoplasmic reticulum (ER), and consists of an outer nuclear membrane (ONM) and an inner nuclear membrane (INM). These membranes are connected via linker of nucleus and cytoskeleton (LINC) complexes, consisting of transmembrane proteins of the INM and ONM joined via intermolecular disulfide bonds in the nuclear envelope lumen [9]. In metazoans, nuclear envelope integrity is further maintained by the nuclear lamina, a protein network underlying the INM [10]. Regulated transport of proteins and RNA across the nuclear envelope is primarily facilitated by multimegadalton NPCs which span the two membranes and are highly conserved among eukaryotes [11–13].

NPCs (Fig. 1B) are made up of multiple copies of approximately 30 different proteins, termed nucleoporins, totaling more than 550 proteins in *S. cerevisiae* and over 1000 proteins in humans [14–16]. A symmetric scaffold of inner and outer rings with eightfold rotational symmetry anchors the NPC in the nuclear membrane at points of fusion between the INM and ONM. Recent studies utilizing integrative structural biology approaches have revealed the high-resolution structure of the NPC scaffold in yeast, *Xenopus laevis* and humans [14–22]. Nucleoporins with intrinsically disordered regions enriched for repeated amino acid sequences containing phenylalanine and glycine residues (FG repeats) are anchored to this scaffold and form a meshwork that constitutes the permeability barrier of the NPC [14,16,23]. Within cells, the directionality of mRNA export is governed by the asymmetric assembly of cytoplasmic filaments and the nuclear basket on the cytoplasmic and nuclear sides of the NPC, respectively [11]. Recent studies have revealed heterogeneity among NPCs found within a single cell, with NPCs located at the nucleolus lacking a nuclear basket [14,24]. Furthermore, the NPC scaffold is dynamic, with significant changes in pore diameter observed in multiple organisms under different conditions [20,25,26]. The functional significance of these variations in NPC architecture remains largely unclear.

The proper maintenance and regulation of nuclear components, in particular the NPC, are of utmost importance for the maintenance of nuclear functions, dynamics, and overall cellular viability [27–30]. While NPC dysregulation, for example, is linked to human diseases including neurodegeneration and cancer, the degradation of nuclear components and their physiological significance have received less attention compared with other organelles such as the ER and mitochondria. In recent years, it has been shown that several autophagic pathways, collectively referred to as nucleophagy, are involved in maintaining nuclear homeostasis. Here, we first review autophagy-mediated degradation of nuclear components in the budding yeast *S. cerevisiae*, which is the best-studied case due to its closed mitosis, and has several autophagy pathways that selectively target and degrade different parts of the nucleus. We later expand to nuclear degradation mechanisms identified in other eukaryotes.

Selective autophagy of nuclear components in *S. cerevisiae*

Selective macroautophagy involves the recognition and sequestration of specific cargo by specialized receptor proteins that selectively recognize the cargo and possess a binding motif for ubiquitin-like proteins of the Atg8/LC3/GABARAP family that are covalently anchored to the lipid phosphatidylethanolamine. This

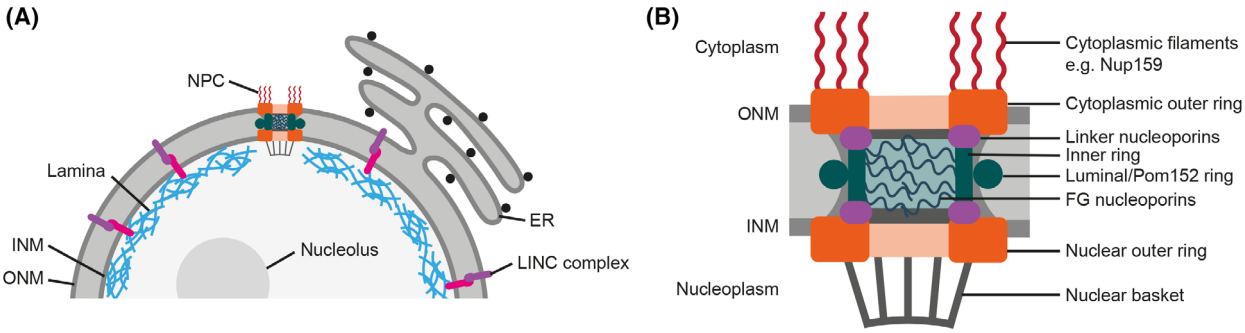

Fig. 1. Organization of the nuclear envelope and NPC structure. (A) Key structural features of the nuclear envelope conserved between eukaryotes. The nuclear lamina is found only in metazoans. (B) Schematic displaying major structural features of the NPC in yeast.

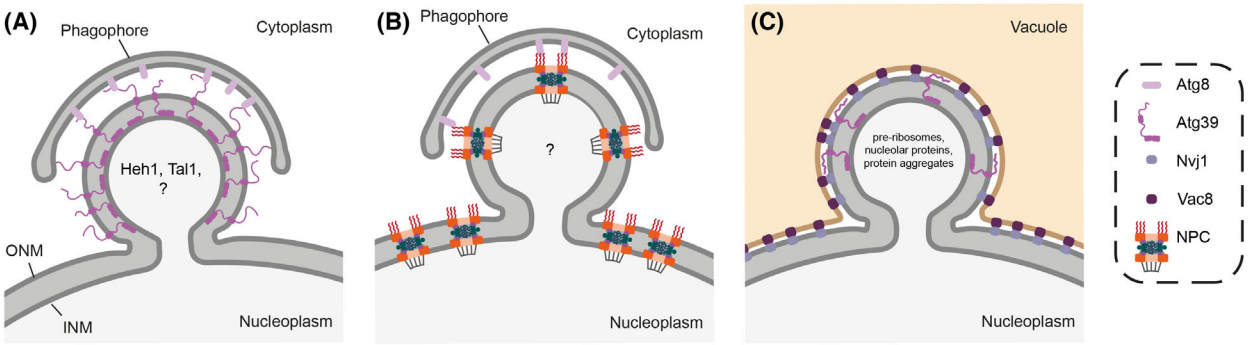

Fig. 2. Nucleophagy mechanisms in *S. cerevisiae*. (A) The first identified nucleophagy receptor in yeast, Atg39 interacts with Atg8 in order to initiate phagophore formation at the nuclear envelope. Additionally, the interactions of Atg39 with both the INM and ONM allow the deformation of the nuclear envelope to form double-membrane-bound NDVs. As yet, cargo specificity for nucleophagy mediated by Atg39, aside from the proteins Heh1 and Tal1, is unclear. (B) A specialized form of nucleophagy for the degradation of NPCs is facilitated by an interaction between the intrinsic autophagy receptor Nup159 and Atg8. Clustering of NPCs at the site of phagophore formation leads to an enrichment of NPCs within the resulting NDVs. (C) Micronucleophagy is distinct from macronucleophagy as it does not involve the formation of a phagophore at the nuclear envelope, but rather budding of the nuclear envelope directly into a degradative compartment, namely the vacuole in yeast. This direct engulfment of the nucleus occurs at nucleus–vacuole junctions and requires the interaction between Vac8 and Nvj1. Atg39 has been implicated in this mechanism but its role remains unclear.

connects the cargo with the growing phagophore. The most commonly found motif, called the Atg8 interacting motif (AIM) or LC3-interacting region (LIR), is often located in intrinsically disordered regions (IDRs) of receptor proteins [31]. In addition, most selective autophagy receptors recruit the autophagy scaffold protein Atg11, which binds to phosphorylated sites on the autophagy receptor protein and synchronizes cargo recognition with phagophore biogenesis [32–34].

One of the first nucleophagy receptors identified in budding yeast was Atg39 [35]. Atg39 is a transmembrane protein with a single transmembrane domain inserted into the ONM [36,37]. Amphipathic helices in its C-terminal domain bind to the INM, linking the two membranes [36,37]. Binding sites for both Atg8 and Atg11 are located in the cytoplasm-facing N-terminal region. A local increase in Atg39 leads to nuclear envelope expansion and remodeling, resulting in the formation of double-membrane nuclear-derived vesicles (NDVs), the mechanisms of which are not yet fully understood (Fig. 2A). However, based on electron tomography data, the current model suggests an initial scission step of the INM, forming a vesicle intermediate within the perinuclear space, followed by a second scission step that releases the NDV into the cytoplasm [36]. Membrane remodeling during this process is likely driven by the amphipathic helices of Atg39, as truncation mutants of these domains result in a loss of degradation of Tal1 and Heh1, identified cargoes of Atg39-dependent nucleophagy [36,37]. The concept of membrane remodeling by autophagy receptors is also found in the case of other organelles such as the ER, where local clustering of reticulon homology domain-containing proteins in the ER membrane initiates ER remodeling [38–40]. In Atg39-dependent nucleophagy, NDV formation is coordinated with autophagosome biogenesis, resulting in autophagic engulfment and subsequent delivery of NDVs to the vacuole. How NDV formation and autophagosome biogenesis are orchestrated is still elusive.

Another possible route for NDVs to enter the vacuole is direct uptake by microautophagy at the nucleus–vacuole junction, specifically referred to as micronucleophagy or piecemeal microautophagy of the nucleus (Fig. 2C) [41–43]. Interestingly, micronucleophagy also appears in some cases to require Atg39 and the core autophagy machinery since the deletion of these proteins blocks autophagic degradation of nucleus–vacuole junction proteins such as Osh1 and Nvj1 [43]. However, this depends on the cargo monitored since not every cargo is strictly dependent on these proteins [44]. It is unclear what role the core autophagy machinery proteins play in micronucleophagy [38–40]. One striking difference between these two nucleophagy pathways is the size of the formed NDVs, with those degraded by micronucleophagy being approximately twice as large as those sequestered during macroautophagy [43]. Since the mechanisms of NDV formation in both pathways are poorly characterized, the cause of this difference in cargo morphology is unknown.

Remarkably, nucleophagy also constitutes an alternative route for nuclear-cytoplasmic transport, enabling the movement of nuclear material across the nuclear envelope. Micronucleophagy preferentially targets

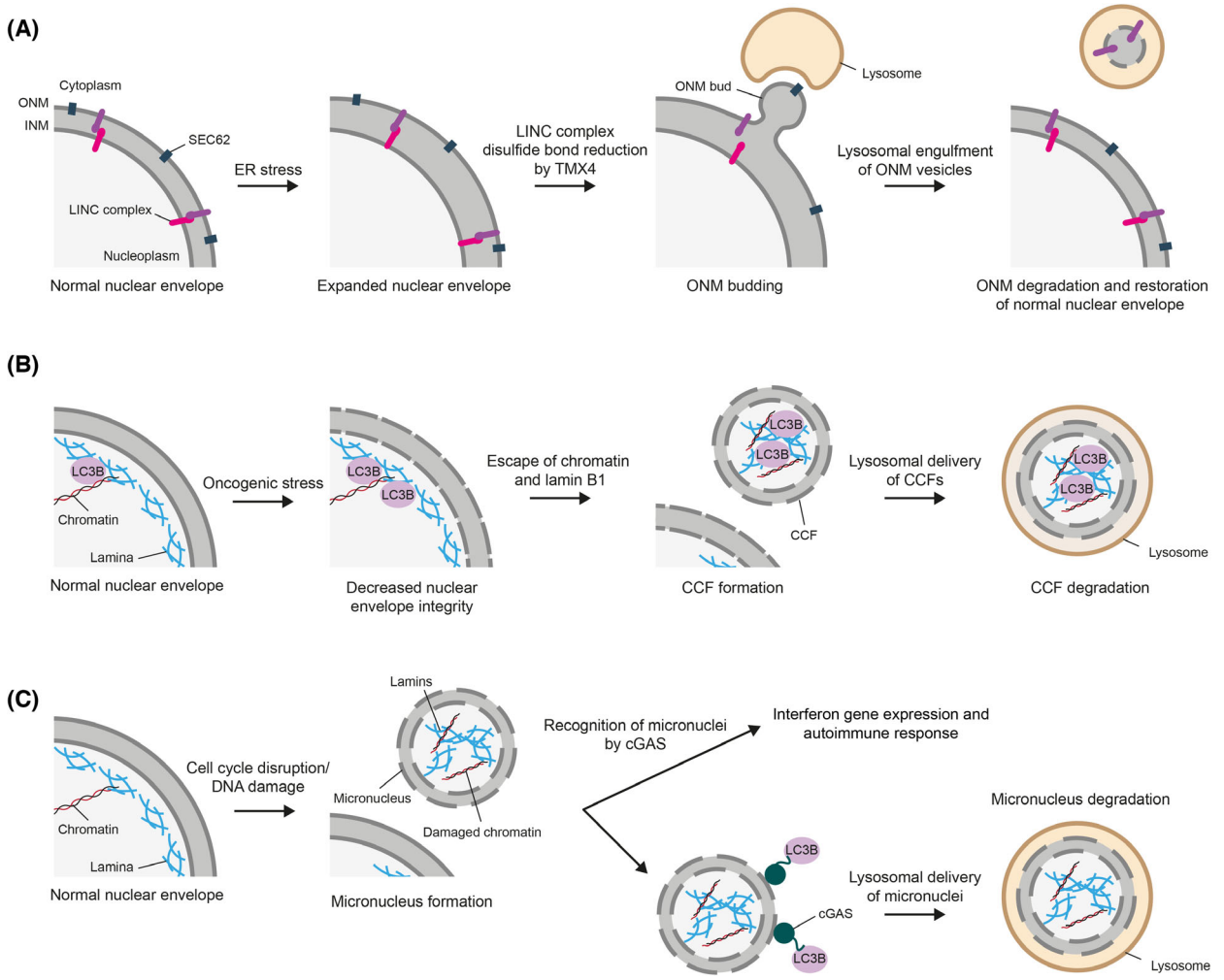

Fig. 3. Selected identified nucleophagy mechanisms in higher eukaryotes. (A) A specialized form of micronucleophagy identified in MEFs degrades the ONM during recovery from ER stress. (B) Lamin B1 is an intranuclear interactor of LC3B-II in human primary IMR90 cells. Upon oncogenic stress, decreased nuclear envelope integrity enables chromatin and lamin B1 bound by LC3B-II to escape the nucleus and form CCFs. The mechanism of CCF delivery to lysosomes for degradation remains unclear. (C) Upon cell cycle disruption or DNA damage in specific cell types, micronuclei containing damaged chromatin are released into the cytoplasm. Recognition of micronuclei by cytosolic cGAS upregulates interferon gene expression via the cGAS/STING signaling pathway. Furthermore, cGAS has been proposed as an autophagy receptor, which may target micronuclei for lysosomal delivery via an uncharacterized mechanism.

nucleolar components including pre-ribosomes [45,46]. In addition, a nuclear-targeted temperature-sensitive variant of firefly luciferase that misfolds at 37 °C is degraded by micronucleophagy, suggesting a quality control function in removal of nuclear protein aggregates [47]. In line with this, the nucleolus has been proposed as a storage compartment for misfolded proteins which are kept in a folding-competent state by the chaperone Hsp70 [48]. Interestingly, micronucleophagy is highly selective, excluding chromosomal DNA, ribosomal DNA (rDNA), and NPCs from degradation via this process [45,46,49,50]. While the mechanism behind this cargo selectivity remains unknown, it has been speculated that, for example, NPCs are too large to enter the nucleus–vacuole junction.

Recently, a third nucleophagy pathway has been uncovered that specifically targets NPCs for degradation and has been named NPC-phagy [51,52]. As shown by electron tomography, NDVs containing NPCs are formed, released, and subsequently sequestered in autophagosomes during NPC-phagy (Fig. 2B) [20,51,52]. Experiments testing the dependence of nucleoporin degradation on Atg11 or the AIM-binding pocket of Atg8 suggested a receptor-mediated degradation pathway, which was only slightly impaired by deletion of *ATG39* [51,52]. Pulldown experiments using

Atg8 as bait revealed direct binding of NPC components via the cytosolic filament protein Nup159. An AIM in an IDR toward the C-terminal half of Nup159 was identified as essential for Atg8 recruitment [51,52]. We showed that mutation in the AIM not only abolished Atg8 binding but also impaired nucleoporin degradation, thus acting as an intrinsic receptor [51]. Moreover, deletion of Nup116, which fails to assemble Nup159 and the cytosolic filament complex at a restrictive growth temperature, also abolished nucleoporin degradation upon induction of autophagy by nitrogen starvation [20]. Interestingly, knockout of the ER-phagy receptor Atg40 showed reduced NPC-phagy, suggesting a potential role for it in the NPC degradation pathway [51,52]. Similarly, the mammalian ER-phagy receptor TEX264 has been described to have a dual role in ER and nuclear quality control [53]. However, Tomioka et al. reported a contrasting finding regarding the dependence of nucleoporin degradation on the AIM of Nup159. According to their results, the AIM appears to facilitate the elimination of unassembled Nup159, a process referred to as nucleoporinophagy, rather than the degradation of whole NPCs. One possible explanation for the different observations is that additional physical interactions play an important role in the efficient turnover of NPCs. Nup159 could potentially possess other auxiliary AIMs, similar to known autophagy receptors, that greatly enhance the affinity of receptor-Atg8 interactions [54,55]. In addition, concurrent interactions with other nucleoporins may drive NPC-phagy. Of note, there was also a difference in the conditions used for induction of autophagy between the two studies, which may also contribute to the observed discrepancies. Further investigations are necessary to address this issue and to gain a comprehensive understanding of the mechanism and physiological significance of NPC-phagy.

The receptor Nup159 contains an N-terminal beta-propeller domain tethering the helicase Dbp5 to the NPC, followed by FG repeats, a pentameric Dyn2-binding domain, and a C-terminal coiled-coil domain. The binding of Dyn2 dimers to Nup159 promotes a stable association of the cytosolic filament complex with the NPC [56,57]. Interestingly, the Nup159 AIM is located adjacent to the Dyn2-binding site and faces toward the cytosolic side of the NPC [51]. It has been shown that binding of Atg8 to Nup159 induces a conformational change in the IDR [58]. Whether Dyn2 influences the Nup159-Atg8 interaction is unknown; however, it is tempting to speculate that Nup159-Dyn2 oligomerization could provide an avidity-driven surface between the Atg8-mediated phagophore membrane and the NPC-containing NDV by recruitment of additional Nup159 proteins. Interestingly, we showed that NPC-phagy is increased in a Nup159-dependent manner by deletion of the y-complex (outer ring) nucleoporins Nup120 and Nup133, which display a constitutive NPC clustering phenotype [59–61]. These results suggest a quality control function of NPC-phagy for NPC assembly or functionality. Future studies will have to address the specificity, selectivity, interplay, and physiology of the individual nucleophagy pathways.

Degradation of nuclear components by autophagy in other eukaryotes

While no homolog for Atg39 has been identified as a nucleophagy receptor in higher eukaryotes, the translocon subunit SEC62 has recently been shown to act as a selective autophagy receptor for the degradation of excess ONM portions during recovery from ER stress in mouse embryonic fibroblasts (MEFs; Fig. 3A). In contrast to macronucleophagy in yeast, where NDVs surrounded by both the INM and ONM are engulfed into autophagosomes, this pathway requires TMX4-catalyzed LINC complex intermolecular disulfide bond reduction to enable ONM-only vesicle formation. Time-course microscopy revealed that these ONM vesicles are engulfed directly into endolysosomes for degradation. This mechanism is thus an example of micronucleophagy in higher eukaryotes [62]. In addition to this SEC62-mediated mechanism, Microtubule-Associated Protein 1 Light Chain 3 Beta (MAP1LC3B/LC3B) has intranuclear binding partners in higher eukaryotes, some of which have been proposed as autophagy receptors [63]. Among these interactors are lamin B1 and lamin A/C, components of the nuclear lamina [10]. In human primary IMR90 cells, lipidated LC3B (LC3B-II) binds lamin B1 and lamin-associated domains of chromatin. Upon oncogenic stress, these interactions, accompanied by elevated expression of autophagy factors, promote lysosomal delivery and degradation of cytoplasmic chromatin fragments (CCFs) containing lamin B1 and chromatin, which bleb from the nuclear envelope (Fig. 3B) [64]. Autophagy of CCFs containing lamin B1 also occurs in senescent human melanocytes [65]. In mice and *Caenorhabditis elegans*, autophagic degradation of lamin B1 is regulated by nesprin-2, which mediates autophagy of other nuclear components, including the nucleolus, to promote germline immortality and prolong somatic cell lifespan [66]. In human breast cancer cells with doxorubicin-induced DNA damage, UBC9-mediated SUMOylation of lamin A/C promotes interaction with

LC3B-II, causing degradation of lamin A/C and leaked chromatin [67].

In addition to nuclear lamina components, the NAD^+-dependent deacetylase SIRT1 is another selective nuclear substrate of autophagy identified in several murine tissues and aged human $CD8^+$ $CD28^-$ T cells. During senescence, decreased SIRT1 phosphorylation likely mediates its increased LIR-dependent interaction with LC3B [68]. Intranuclear Atg8-binding partners are also reported in plants. Cellular levels of the transcription factor Heading date 1 (Hd1) in *Oryza sativa* are mediated by an intranuclear AIM-dependent interaction with Atg8. AIM mutation increases cellular Hd1 level, contributing to delayed flowering, specifically under dark conditions [69]. Furthermore, immune response-associated degradation of C1 protein from tomato leaf curl Yunnan virus residing in the nucleus of *Nicotiana benthamiana* and *Solanum lycopersicum* is dependent on C1 interaction with Atg8. In this case, the Atg8-cargo interaction both targets the C1 protein for degradation via autophagy and enables C1 export from the nucleus via the Xpo1 export pathway, presumably to access the cytoplasmic autophagy machinery [70].

In the absence of a directly detected interaction between Atg8/LC3/GABARAP family members and a nuclear binding partner, autophagy of nucleus-derived material has still been observed. During open mitosis, micronuclei containing chromatin surrounded by aberrant nuclear envelope form when chromosomal fragments fail to be incorporated into daughter cell nuclei [71]. In U2OS cells, chemical cell cycle perturbation increases the frequency of micronuclei, some of which colocalize with LC3B, the lysosome marker LAMP2 and the autophagy receptor p62/SQSTM1, which binds ubiquitinated proteins [72]. Chromatin in these micronuclei is positively stained for the DNA damage marker γH2AX and perturbation of autophagy machinery reduces genomic stability in these cells, suggesting autophagy of micronuclei contributes to genome maintenance and protection against DNA damage [73]. Micronucleus-like structures containing damaged chromatin and lamins also form in a MEF cell model for Aicardi–Goutières syndrome (AGS), in which deletion of the ribonuclease RNaseH2 increases aberrant incorporation of ribonucleotides into DNA. In this model, autophagy inhibition increases the abundance of micronuclei, which colocalize with LC3B. This increase in micronuclei correlates with upregulated cGAS/STING-mediated expression of interferon-stimulated genes, which is a typical autoimmune response in AGS [74,75]. cGAS interacts with LC3B via a LIR and is a potential selective autophagy receptor for these micronuclei (Fig. 3C) [76]. A link between lysosomal delivery of nuclear content and autoimmunity is also observed in MEFs, where deletion of *ATG5* leads to an increase in extranuclear DNA which is not delivered to lysosomes, and a resulting STING-dependent autoimmune response [77]. Autophagy may therefore reduce the occurrence of autoimmune responses against cytoplasmic accumulations of nuclear components. In addition to the degradation of micronuclei containing both chromatin and lamins, autophagy is implicated in clearance of extranuclear chromatin apparently unassociated with nuclear lamina. In lamin A mutant ($Lmna^{H222P/H222P}$) MEFs with compromised nuclear envelope integrity, extranuclear damaged chromatin accumulates and colocalizes with autophagosome biogenesis factors and LAMP2, a lysosomal membrane protein, implicating autophagy in its removal [78]. Furthermore, upon arginine starvation of prostate cancer cells harboring a mutation in argininosuccinate synthetase (ASS1), reactive oxygen species accumulate due to mitochondrial dysfunction, leading to DNA damage. Damaged DNA is extruded through the nuclear envelope, which is remodeled under these conditions, and is similarly targeted by the autophagy machinery for degradation [79].

In extreme cases, autophagy degrades entire nuclei, such as during the formation of the appressorium, a structure required for virulence, in the rice blast fungus *Magnaporthe oryzae*. The absence of nucleus–vacuole junctions in *M. oryzae*, in addition to a requirement for Atg1 and Atg4, implicates macroautophagy in this process [80]. Additional mechanisms for macroautophagy-mediated degradation of entire nuclei have been described in basal cells of hyphae for hyphal growth in the filamentous fungus *Aspergillus oryzae*, and during hyphal fusion in pathogenic *Fusarium oxysporum* [81,82]. A better-characterized mechanism for entire nuclear autophagic degradation occurs during sexual reproduction in *Tetrahymena thermophila*. Following chromatin condensation in the somatic macronucleus of parental cells, changes in protein and lipid composition of the ONM are associated with Atg5 and Atg8 recruitment, promoting lysosome localization to the nuclear periphery and acidification of the nuclear interior, resulting in total nuclear degradation [83–86]. The nature of the autophagosomal structures involved is unclear. Additional membrane sheets are not observed at the nuclear periphery, suggesting that canonical autophagosome formation and autophagosomal engulfment of nuclear portions does not occur, despite the involvement of a Vps34 homolog [83,87]. Autophagy of entire nuclei also occurs in higher eukaryotes. In *C. elegans*, the number and size of intestinal nuclei

decrease over time. Electron micrographs of remaining nuclei show additional perinuclear membranous structures, speculated to be autophagy intermediates [88]. In terminal differentiation of mammalian keratinocytes, organelles including the nucleus are lost during autophagic cell death. Nuclear degradation is attributed to autophagy due to LC3B recruitment to the nuclear envelope, an increase in autophagy factor expression at late stages of differentiation and colocalization of extranuclear DAPI-stained puncta with LC3B in differentiating epidermis of Atg7$^{-/-}$ transgenic mice [89,90]. Phosphorylation of lamin A/C by AKT1 is required for effective nuclear degradation, presumably facilitating nuclear blebbing due to reduced nuclear envelope integrity [91]. Retention of nuclear material in differentiated keratinocytes is linked to lesion formation in psoriasis and decreased expression of autophagy factors in psoriatic lesions suggests a possible link between nuclear autophagy defects and disease manifestation [89–91]. It should be noted that a noncanonical autophagy-like mechanism is also responsible for the degradation of entire uncellularized nuclei during yeast gametogenesis. In this case, rather than delivery of portions of the nucleus to degradative compartments, nuclear destruction is facilitated by vacuole permeabilization and release of degradative enzymes into the cytoplasm of the mother cell during sporulation [92].

Concluding remarks

Highlighted nucleophagy mechanisms across eukaryotes demonstrate diversity in their physiological causes, consequences, and disease associations. However, mechanistic details for these processes are severely lacking, even presenting challenges when defining them as micro- or macroautophagic. The example of complete nuclear degradation in *T. thermophila* also suggests the existence of nucleophagy mechanisms distinct from these two forms of autophagy. Further mechanistic investigation of the formation of nuclear autophagic cargo, such as NDVs, micronuclei and CCFs, and their interactions with autophagic structures, as well as systematic identification of receptors involved in nuclear autophagy will be essential to increase our understanding of the nuclear autophagy landscape across eukaryotic species. Also, to date no NPC-phagy pathway in higher eukaryotes has been described. In analogy to yeast, it will be important to investigate this in postmitotic cells such as neurons, which could potentially rely on such mechanisms for nuclear quality control. Moreover, by now it is clear that many membrane remodeling events occur at the nuclear envelope across different organisms, which leads to the formation of NDVs [93]. This allows various different cargoes such as viruses, RNA granules, and proteins to escape the nucleus and be delivered to the cytosol. It is expected that many of these pathways are potentially linked to autophagy for quality control purposes, as such nuclear egress coupled to autophagy provides an elegant solution for waste removal from a highly regulated intracellular organelle.

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