CHAPTER ONE

Nucleophagy mediators and mechanisms

Margarita-Elena Papandreou[a,b], **Nektarios Tavernarakis**[a,b,*]
[a]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology—Hellas, Heraklion, Greece
[b]Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece
*Corresponding author: e-mail address: tavernarakis@imbb.forth.gr

Contents

Abstract

Nuclear recycling is essential for cell and organismal homeostasis. Nuclear architecture perturbations, such as nuclear loss or nuclear enlargement, have been observed in several pathological conditions. Apart from proteasomal components which reside in the nucleus, specific autophagic proteins also shuttle between the nucleus and the cytoplasm. Until recently, only the microautophagic degradation of nuclear components had been described. Recent studies, dissecting nuclear material recycling in organisms ranging from yeast to mammals, provide insight relevant to other forms of nucleophagy and the mediators involved. Nucleophagy has also been implicated in pathology. Lamins are degraded in cancer through direct interaction with LC3 in the nucleus. Similarly, in neurodegeneration, Golgi-associated nucleophagy is exacerbated. The physiological role of nucleophagy and its contribution to other pathologies remain to be elucidated. Here we discus recent findings that shed light into the molecular mechanisms and pathways that mediate the autophagic recycling of nuclear material.

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Abbreviations

ALS	amyotrophic lateral sclerosis
AMPK	AMP-activated protein kinase
BNIP3	BCL2 and adenovirus E1B 19-kDa interacting protein 3
ER	endoplasmic reticulum
FUNDC1	FUN-14 domain containing protein 1
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
HDAC	histone deacetylase
Hmo1	high-mobility group protein 1
LN	late nucleophagy mTOR mammalian target of rapamycin
NPC	nuclear pore complexes
Nvj1	nucleus-vacuole junction
PE	phospahtidylethanolamine
PEX5	pexophagy 5
PI3K	phosphatidylinositol-3-kinase
PMN	macronucleophagy
PNS	peripheral nerve syndrome
RNP	ribonucleoprotein complex
SAR	selective autophagy receptor
SCA	spinocerebellar ataxia
Sirt1	sirtuin 1
TDP-43	TAR DNA-binding protein 43
TORC1	target of rapamycin complex 1
UBC	ubiquitin-C
UBLs	ubiquitin like proteins
ULK1	Unc-51 like autophagy activating kinase
Vacp8	vacuolar protein 8

1. Introduction: General and selective autophagy

Autophagy from the Greek words "auto," self, and "phagy," eating, is a physiological homeostatic mechanism in all eukaryotes. It is the main catabolic pathway of the cell and breaks down macromolecules such as lipids, proteins and organelles, via the main degradative compartment, the lysosome. Although it was initially considered a bulk degradation pathway, recent evidence highlights its highly selective nature. It can be subdivided into three mechanisms: macroautophagy, thereby referred to as autophagy, microautophagy and chaperone-mediated autophagy.[1]

Autophagy entails the formation of a double membranous vesicle, the autophagosome, which encircles the cargo to be degraded. Specificity is accomplished through recruitment of selective autophagy receptors (SARs).

Yeast Atg19 and mammalian p62/sequesterome are examples of SARs which mediate the interaction between core autophagic components and substrates.[2] Briefly, initiation of autophagy involves yeast Atg1/Unc-51-like, autophagy activating kinase (ULK1), ATG13 and FIP200 which trigger phagophore formation followed by vesicle nucleation through class-III phosphatidylinositol-3-kinase (PI3K) complex which comprises VPS34, VPS15, Beclin-1.[3] SARs interact with ubiquitin like proteins (UBLs), small globular proteins, such as the yeast Atg8/mammalian LC3, required for autophagosomal biogenesis and recruited to the autophagosomal membranes through phosphatidylethanolamine (PE) conjugation, which causes the conversion of LC3-I to LC3-II. The Atg5/Atg12 complex also contributes to the maturation of the autophagosome. Autophagy of specific organelles, lipids or proteins is coordinated by multiple signaling pathways under various environmental stresses. An Atg8-interacting motif (AIM)/LC3-interacting region (LIR) is found in SARs and targeted by Atg8/LC3. The general core motif, which includes W/F/Y-X-X-L/I/V, with negatively charged residues upstream of the motif triggers a higher affinity interaction as well as post-translational modifications, such as phosphorylation.[4] In addition, these adaptor proteins contain domains such as the UBA motif, which recognizes ubiquitinated proteins for degradation. K27-linked mono-ubiquitination and K63 poly-ubiquitination are characteristic examples of tagging for autophagosomal recycling.[5] This allows tight regulation of transport of autophagic substrates for lysosomal degradation.

Different selective autophagy receptors exist depending on the substrate to be degraded. Protein aggregate degradation, aggrephagy is mediated by SARs such as p62, optineurin, NBR1 and NDP52.[6–8] Organellophagy, the autophagic recycling of organelles can occur with the concerted action of the SARs referred to above, and more specific ones such as the BCL2 and adenovirus E1B 19-kDa interacting protein 3 (BNIP3), and FUN-14 domain containing protein 1 (FUNDC1) for mitophagy.[9,10] Mutations in SARs can lead to severe phenotypes such as metabolic disorders, neurodegenerative and age-related disease.[11] In mitophagy, there are several receptors modulating mitochondrial degradation. Mutations in the PINK1 and Parkin proteins involved in the ubiquitination of outer mitochondrial membrane proteins that are then recognized by SARs such as p62, OPTN and NDP52, cause Parkinson's disease.[12] Optineurin mutations have been associated with amyotrophic lateral sclerosis (ALS).[13] In addition, mutations in the PEX5 gene cause the fatal cerebro-hepato-renal Zellweger syndrome. PEX5 encodes a pexophagy-specific receptor, which when

ubiquitinated interacts with p62, and is transported to the autophagosome.[14,15] Therefore, different protein receptors/adaptors found either on the organelle, or recruited under stress, are required to efficiently regulate autophagic degradation.

1.1 The nucleus

Nucleophagy, the recycling of the nucleus by autophagy, has not been investigated in depth although the nucleus is the most prominent organelle of the cell. To discern potential receptors and pathways, which control degradation of specific nuclear components and compartments, a meticulous study of nuclear structure is required. The phospholipid bilayer of the nuclear membrane is a barrier tightly controlling communication between the cytoplasm and the nucleoplasm. The nuclear membranes are continuous with those of the endoplasmic reticulum (ER) but significantly differ in protein composition. Nucleoporins form large nuclear pore complexes (NPCs) for bidirectional movement of ribonucleoprotein complexes (RNPs), RNA and proteins between the nucleus and the cytoplasm.[16] The inner nuclear membrane of the nuclear envelope consists of integral proteins that interact with the nuclear lamina (nucleoskeleton) and chromatin, thus, influencing chromatin localization and gene expression. Examples include emerin, SUNs, lamin B receptors (LBRs) that interact with B-type lamins and heterochromatin protein 1 (HP1), which, in turn, interacts with chromatin. SUN proteins interact with nesprins, the outer nuclear membrane proteins (ONMs) that connect the nucleus with the cytoskeleton, and together form the linker of the nucleoskeleton-cytoskeleton complex (LINC). ONMs share a small Klarsicht, ANC-1, Syne Homology (KASH) domain. They regulate nuclear migration, cell polarization and maintain nuclear shape. However, many of the nuclear envelope proteins remain largely uncharacterized. Inner nuclear components include the nucleolus, nuclear bodies, such as the promyelocytic leukemia PML bodies, DNA, RNA, and proteins that shuttle nucleocytoplasmically.

1.2 Nucleophagy in yeast

Yeast cells perform micronucleophagy as well as macronucleophagy. Nucleophagy was first described in *Saccharomyces cerevisiae*, in two forms, piecemeal macronucleophagy (PMN) and late nucleophagy (LN).[17,18] Apart from inducing general autophagy, TORC1 (target of rapamycin 1) inactivation triggers nucleophagy through the Nem1/Spo7-Pah1 axis, which properly localizes micronucleophagy component Nvj1 and nucleophagy receptor Atg39.[19]

PMN occurs under nutrient-rich conditions, after rapamycin treatment, or short nutrient and nitrogen deprivation. Mechanistically, the lytic vacuole and the nucleus initiate direct interaction with the formation of tight nuclear-vacuole junctions (NVJ). Subsequently, ONM and nuclear ER protrusions develop, which then become ER-derived vesicles encircled by the lytic vacuole.[20] Then, vesicles are pinched off the nucleus; nuclear-derived membranes are fused at multiple points with the vacuole membrane for the release of substrates to be degraded by lytic enzymes. The outer nuclear membrane nucleus to vacuole protein 1 (Nvj1p) and vacuolar protein 8 (Vacp8) mediate micronucleophagy, emphasizing the importance of autophagy selectivity, even in lower eukaryotes. Vacp8 together with Apg13p mediate cytoplasm-to-vacuole targeting pathway.[21] Although it is suggested that core autophagic genes are implicated in this process., recently a new study reveals that microautophagy does not require macroautophagic proteins, but endosomal sorting complex proteins instead such as Vph1, Pho8 and Vps27.[22] Of note, RNA non-selective degradation has been reported after nitrogen starvation.[23] However, it is not known whether nuclear RNA is directly degraded or whether cytoplasmic RNA is recycled by general autophagy after transported to the cytoplasm. Autophagic cargo of PMN includes nuclear envelope components, the granular nucleolus, containing pre-ribosomes, without nuclear pore complexes and spindle pole bodies, and RNA.[24] CLIP and cohibin are mediators of nucleolar protein degradation by spatially separating them from ribosomal DNA that is not degraded, tethering them on the ONM, and directing them proximal to NVJs.[25] The complex of condensin, Rpd3–Sin3 histone deacetylase (HDAC), and high-mobility group protein 1 (Hmo1) are essential for ribosomal DNA condensation.

PMN and LN are almost always mutually exclusive, both temporally and spatially. LN which was later identified as macronucleophagy, is only induced after prolonged (18–24 h) nitrogen starvation, and, in contrast to PMN, can trigger nuclear shape alterations, indicating accumulation and/or aggregation of nuclear cargo that is not degraded.[17] This can be distinguished by two differential reporters Nvj1p-EYFP, for the former and n-Rosella for the latter.[26] LN requires Nvj1p and Vac8p and other autophagy-related proteins, such as Vps34p, Vps15p, Atg6p, and Atg14p or Atg11p.

Recently, another mode of ERphagy and nucleophagy has been described in yeast.[27] Specifically, the Atg39 receptor, found in perinuclear ER is required for macronucleophagy, while Atg40 mediates ERphagy while contributing to macronucleophagy. Moreover, it was shown that both

Table 1 Nucleophagy factors and pathways.

Factors	Piecemeal nucleophagy	Macronucleophagy	Mammalian nucleophagy
Macroautophagy genes	–	*atg1*, *atg2*, *atg3*, *atg4*, *atg5*, *atg7*, *atg8*, *atg9*, *atg10*, *atg12*, *atg13*, *atg16*, *atg18*, *atg23*, *atg29*, *atg31*	*Atg7*, *Lc3*
Receptor	Nvj1p, Vac8p	Vac8p, Atg39, Atg40	–
Substrates	Nuclear envelope components, granular nucleolus, pre-ribosomes, spindle pole bodies, RNA	Nuclear envelope components, granular nucleolus, pre-ribosomes, spindle pole bodies, RNA, Hmg1, Src1, Nop1	Lamin A, lamin B2
Pathway/Inducer	Short nitrogen starvation, TORC1 inactivation, Nem1/Spo7-Pah1 axis	Prolonged nitrogen starvation, TORC1 inactivation, Nem1/Spo7-Pah1 axis	DNA damage, oncogenesis
Phenotype	–	Nuclear shape alterations	Cell death, neurodegeneration, senescence

Atg39 and Atg40 are substrates of macroautophagy as genetic inhibition of macroautophagy causes an increase of their levels. Substrate degradation by this key regulator includes ONM and INM proteins, Hmg1, Src1, and nucleolar protein Nop1. However, no exact homolog has been found in mammals although functionally similar SARs have been identified.[18] A comparison between PMN and LN is shown in Table 1.

1.3 Interplay between autophagy and the nucleus in mammals

The crosstalk between the autophagic machinery and nuclear components in mammals has recently drawn much attention. The main autophagosomal membrane protein LC3 is stored in the nucleus under basal conditions in an acetylated form.[28] Deacetylation occurs selectively under starvation at

residues K49 and K51 by sirtuin 1 (Sirt1). LC3 is then transported to the cytoplasm by DOR, where it can be conjugated in preautophagosomal membranes through conversion to LC3-II. It is still unclear whether LC3 localization in the nucleus has other functional implications, apart from storage purposes.

Selective autophagy receptors and adaptors are also localized in the nucleus, the function of which remains largely unexplored (Fig. 1). p62, which continuously shuttles between the nucleus and the cytoplasm, together with large protein ALFY, synergize to target ubiquitinated proteins to PML bodies.[29] Moreover, upon stress, ALFY becomes localized with p62 to ubiquitin-positive bodies in the cytoplasm. In addition, nuclear ATG5 and ATG7 can regulate p53 activation and cell cycle progression as well as autophagy.[30,31]

Filamentous fungi contain multinucleate cells called hyphae that proliferate from the hyphal tip. Basal cells undergo nucleophagy of whole nuclei as well as pexophagy and mitophagy.[32] It has been proposed that autophagosomes for nuclei are specific as they are much larger. Colony growth is inhibited upon perturbation of autophagy, indicating that autophagy and potentially nucleophagy-derived catabolic products are

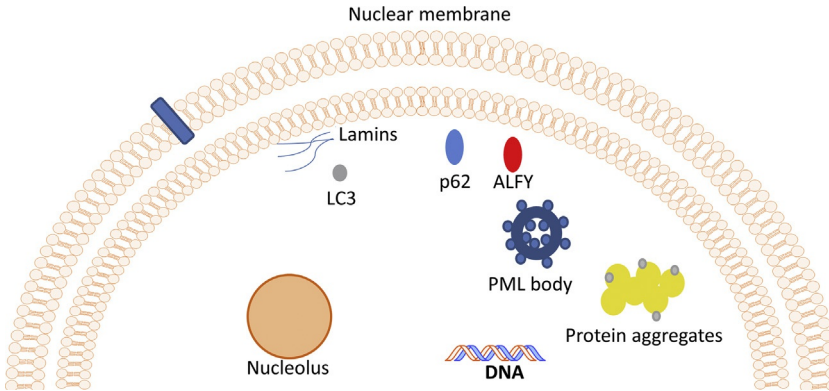

Fig. 1 Autophagy and the nucleus. LC3 (gray), when activated by deacetylase Sirt1, is transported to the cytoplasm. LC3 can transport cargo, such as lamin B2 (blue lines), to autophagosomes during oncogenesis. Similarly, Golgi-mediated lamin A degradation occurs during neurodegeneration. P62 (blue) and ALFY (red) synergize to transport ubiquitinated proteins (yellow) to PML bodies (blue circle). DNA is degraded in the lysosome by Dnase2a, but the path to degradation is still poorly understood. Outer nuclear components (blue rectangle) can also be degraded by interacting with core autophagic components localized in the cytoplasm. Parts of the nucleolus (orange) are degraded by microautophagy, in yeast. Whether this also occurs in higher organisms is unknown.

required for survival and proliferation under starvation, given that nuclei contain large amounts of phosphorus and nitrogen. Thus, nucleophagy is likely an energy source for survival, in this case. Nucleophagy has also been observed during differentiation of skin epidermal keratinocytes. As these cells migrate from the epidermis to the granular layer, they convert into corneocytes which are anucleated cells.[33] During this process, perinuclear LC3 positive vesicles containing p62 and HP1α are localized close to lamins.

Nucleophagy has been shown to play an important role in an oncogenic setting. Electron microscopy has revealed specific nuclear alterations in shape, size and patterns.[34] Chromosomes, protein, and PML bodies are relocalized, while the largest structure of the nucleus, the nucleolus, can become enlarged to meet the excessive protein synthesis needs of transformed cells.[35] Autophagy induces cellular senescence after administration of DNA damaging agents, such as etoposide. The first unequivocal indication of nucleophagy in mammals was obtained during oncogenesis by KRAS, in primary human cells.[36] In this setting, nuclear LC3 directly interacts with lamin B1 through a LIR motif. They are then transported with heterochromatic regions to the cytoplasm for lysosomal degradation (Fig. 2).

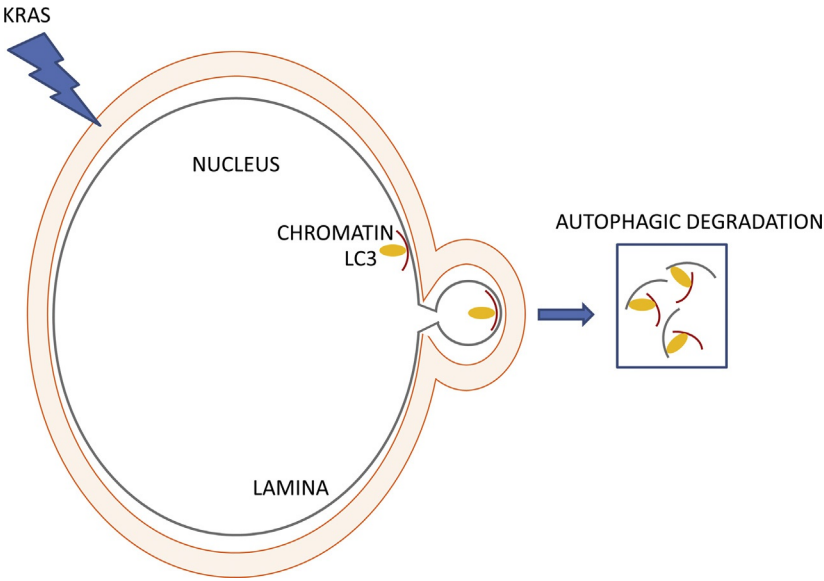

Fig. 2 Mammalian nucleophagy in oncogenesis. LC3 (yellow) interacts with the nuclear lamina (gray), specifically lamin B2 which contains a LIR motif, and transports it together with chromatin fragments (red). A nuclear bulge is formed, which is pinched off and transported to the cytoplasm for autophagic degradation.

Interestingly enough, starvation or mTOR inhibition does not elicit this response, underlining the specificity of this mechanism. Genetic inhibition of ATG7 or mutations in the LIR motif lead to failure of lamin B degradation and subsequent premature senescence, although the exact mechanism has not yet been elucidated. In photoaging, lamin B has been shown to increase and thus act as a biomarker after UV irradiation.[37] Even more recently, SUMOylation of laminA/C by nuclear ubiquitin-C 9 (UBC9) has been shown to be critical for DNA leakage and lamin degradation.[38] Thus, nucleophagy could serve a protective function to restrict DNA replication and proliferation of cancerous cells. Self DNA has been shown to cause autoimmune disorders when not cleared out by transport to the cytoplasm and degradation in the lysosome. Dnase2a or autophagy deficiency causes extra nuclear DNA accumulation and induces inflammation via the STING DNA sensing pathway.[39]

Defects in general and selective autophagy have been implicated in neurodegenerative disease, whereby protein and organelle aggregation is a direct result of defective autophagy. Polyglutamine (PolyQ) diseases are triggered by expansion of CAG repeats in the genes encoding PolyQ proteins. Dentatorubral-pallidoluysian atrophy which is characterized by ataxia, dementia and epilepsy, is caused by atrophin mutations, and manifests a pathological form of nucleophagy.[40,41] At the cellular level, canonical autophagy is stalled, while Golgi membrane degradation and nuclear breakdown occurs via nucleophagy based lamin B degradation and excretion. Concomitantly, there is reduction of LC3-I/II conversion, increase of p62, DNA damage, and senescence. Blockage of autophagy with simultaneous induction of alternative degradative pathways causes nuclear architecture disruption, nuclear collapse and cellular atrophy. Apart from specialized cases in fungi, nucleophagy has been shown to occur in pathological, rather than physiological conditions. Both in a cancerous and neurodegeneration setting, nucleophagy is recruited to clear out nuclear aggregates, damaged DNA, or protein aggregates. Whether, basal nucleophagy occurs, albeit at low, non-pathological levels, remains to be elucidated.

1.4 Nuclear morphology alterations and disease

Nuclear abnormalities and nuclear-derived pathology have been observed in disease. However, in many cases, the mechanisms underlying such phenotypes have not been delineated. Complete nuclear loss or reduction in DNA copy number in the intestine and other cells has been observed during aging,

in the nematode *Caenorhabditis elegans*.[42] Long-lived *daf-2* mutant animals display delayed manifestation of such phenotypes, indicating at least partial regulation of the underlying processes by the insulin/IGFI signaling pathway.[43] It has been suggested that autophagy degrades nuclei during aging, possibly to supply nutrients. Nuclear size alterations, or nuclear malformations, which could be a direct or indirect consequence of nucleophagy, occur in pathological conditions such as malignancy which could be the result of DNA, nuclear protein accumulation or nuclear envelope recycling dysregulation.[44]

A multitude of nuclear envelopathies have been identified. Mutations in *LMNA* can affect different tissues causing a variety of pathologies. Examples include the Emery-Dreifuss muscular dystrophy, and dilated cardiomyopathy, which can also be caused by mutations in emerin and Nesprins.[45–47] Apart from striated muscle pathology, lipodystrophy manifests in patients with *LMNA* and *LMNB2* defects. Other diseases include peripheral nerve syndrome (PNS) disorders, bone abnormalities and progeria syndromes. Nesprin 1/2 mutations have also been linked to cardiomyopathies. Indeed, myocytes from such patients have enlarged nuclei. Senescence can also arise upon perturbation of Nesprin function, which could be attributed to the compartmentalization of the extracellular signal-regulated kinase 1/2 (ERK1/2) to PML bodies.[48]

Defects in nucleolar shape and size are prominent during aging and have been proposed as biomarkers of premature aging. Reproductively aged mice exhibit larger nucleoli, thus, lower oocyte quality, which leads to decreased fertility.[49] This extends to different tissues, both in cell cultures and in *C. elegans*. Nucleolar size is predictive of cell/organismal lifespan.[50,51] *daf-2*, *eat-2*, *ife-2* and *glp-1*, long-lived mutant strains display smaller nuclei compared to wild-type worms. Thus, these signaling pathways extend lifespan partially through restricting nucleolar expansion and ribosomal biogenesis. Complementarily, in Hutchison-Gilford progeria syndrome, cells contain larger nucleoli, indicating increased ribosome biogenesis and protein translation.[50] Nucleolar size is not merely a biomarker of aging. Reducing fibrillarin protein production, a key nucleolar protein, extends lifespan. In addition, mutant prelamin A, progerin, which causes progeria syndrome increases nucleolar size. Rapamycin, which induces macroautophagy, has been shown to ameliorate cellular phenotypes in Hutchison-Gilford progeria syndrome by degrading the mutant protein.[52]

Perturbed nucleophagy could be the underlying cause of a variety of nuclear inclusion body diseases. Accumulation of mutant huntingtin, ataxin and androgen receptor cause Huntington's disease, spinocerebellar ataxias

(SCAs), and spinobulbar muscular dystrophy, respectively. Multiple muscle atrophy is characterized by filamentous nuclear aggregates associated with the nucleolus or the inner nuclear membrane.[53] Inefficient clearance of these proteins causes neurological defects and neuronal cell death. Nuclear GAPDH, when associated with DNA, upon oxidative stress, blocks DNA damage repair and triggers cells death.[54] Importantly, the AMP-activated protein kinase (AMPK) activates GAPDH upon glucose starvation, which in turn activates Sirt1. Sirt1 has been shown to deacetylate nuclear LC3 triggering its cytoplasmic shuttling. This molecular pathway could be pharmacologically exploited to trigger nuclear autophagy. Identifying inducers of nucleophagy could potentially allow clearance of excess pathological aggregates, particularly because nuclear pores further restrict large protein transport, when protein aggregates are formed that can themselves perturb nuclear pore function.[55]

2. Conclusion and future perspectives

Selective autophagy of nuclear components occurs in organisms ranging from simple single cell eukaryotes to humans. Substrates can be RNA, diffuse proteins or components of the nuclear envelope, and the nucleolus. In yeast, different types of micronucleophagy exist under both basal and nutrient deprivation conditions. In mammals, although there are indications of nucleophagic events under physiological conditions, nucleophagy has mainly been associated with pathology. For example, during oncogenesis and neurodegeneration, nucleophagy targeting lamins is prominent, resulting in cellular senescence and neuronal death, respectively. Identification of potential instigators of macronucleophagy in physiology would spur new research directions in the field. Apart from neurodegeneration caused by excess nucleophagy, an additional open question relates to the consequences of nucleophagy deficiency. Molecular pathways selectively inducing this autophagic process could be therapeutic targets for a multitude of diseases linked to the formation of nuclear aggregates. Hence, given that the nucleus safeguards the genetic material in addition to controlling its expression via mRNA transcription and translation, it is important to better understand the contribution of nucleophagy in maintaining nuclear homeostasis, both in the context of physiology and pathology. Nucleophagy may well be a protective mechanism against age-related disease due to nuclear dysfunction and a potential therapeutic target.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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