

The intricacy of nuclear membrane dynamics during nucleophagy

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The cell nucleus is an organelle bounded by a double-membrane which undergoes drastic reorganization during major cellular events such as cell division and apoptosis. Maintenance of proper nuclear structure, function and dynamics is central to organelle vitality. Over recent years growing evidence has shown that parts of the nucleus can be specifically degraded by an autophagic process termed nucleophagy. The process is best described in the yeast, *Saccharomyces cerevisiae*, where piecemeal microautophagy of the nucleus or nucleophagy (micronucleophagy) requires direct interaction of the nuclear membrane with that of the vacuole (the yeast lytic compartment). Here, we review the process of nucleophagy in the context of nuclear membrane dynamics, and examine the evidence for autophagic degradation of the nucleus in mammalian cells. Finally, we discuss the importance of nucleophagy as a 'housecleaning' mechanism for the nucleus under both normal and disease conditions.

Introduction: The Need for Nuclear Turnover

The nucleus is a site of a number of essential metabolic activities relating to maintenance and expression of the genome. Such activities include: DNA replication, recombination and repair, gene transcription, RNA processing and ribosome subunit maturation and assembly.^{1,2} In order for these essential activities to be carried out in a correct and efficient manner the corresponding machinery must be maintained. Consequently, it is to be expected there are processes within cells which act to 'repair' nuclear damage through the coordinated removal of damaged non-functional components, or those surplus to requirement. A growing body of evidence indicates that parts of the nucleus can be specifically degraded by nucleophagy (micronucleophagy), an autophagic process involving localized changes in membrane organization and dynamics. Here, we discuss nucleophagy in this context as well as its role in 'housecleaning' during nutrient

limitation and under various physiological and pathological conditions.

Nuclear Structure, Function and Compartmentalization

The nucleus is not only the largest membrane-enclosed organelle in eukaryotic cells, but arguably the most elaborate. Two main structures make up the metazoan (including mammalian) nucleus. The first of these is the nuclear envelope (NE) that acts as the interface between the nucleus and the rest of the cell enclosing the contents of the nucleus.^{3,4} The second is the nuclear lamina, a dense but fenestrated network composed of intermediate filaments made of lamins and lamin-associated (e.g., emerin) proteins underlying the inner surface of the NE. The NE is required for maintenance of nuclear shape, spacing of nuclear pore complexes (NPCs), organization of heterochromatin, DNA replication and regulation of transcription factors (Fig. 1).⁵⁻¹¹ Certain organisms, such as fungi or plants lack lamins¹² and consequently lack a nuclear lamina.¹³ Plants contain coiled-coil proteins, apparently unrelated to lamins, which serve as nucleoskeleton components.^{12,14,15} Coiled-coil proteins have been implicated also as nucleoskeleton components in non-metazoans, notably yeast and *Trypanosoma brucei*.^{12,16,17}

The NE is comprised of two concentric, closely opposed lipid bilayers, the outer and inner nuclear membranes (ONM and INM, respectively).^{4,18} The ONM is continuous with the rough endoplasmic reticulum (rER), studded with ribosomes and functions in secretion and lipid biosynthesis. The ribosome-free INM faces the viscous nucleoplasm, and contains a unique spectrum of integral and membrane-associated proteins that provide binding sites for the nuclear lamina and chromatin. The ONM and INM are separated by a luminal space but become contiguous at sites of high membrane curvature occupied by the NPCs (Fig. 1). NPCs exclusively mediate and regulate nucleocytoplasmic trafficking, the signal-dependent, bidirectional trafficking of macromolecules between the nucleus and cytoplasm which is crucial for both gene expression and chromosomal maintenance.^{7,18-20}

The interior of the nucleus lacks membrane-bound subcompartments, but is organized into domains usually referred to as "subnuclear structures" (also often referred as "nuclear organelles" or "nuclear compartments") with highly specialized functions and containing a unique complement of proteins and RNA molecules. This subnuclear organization is promoted and maintained

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through protein-protein and protein-nucleic acid interactions. The subnuclear structures [e.g., nucleolus, chromosome territory, the Cajal body (CB), promyelocytic leukemia oncoprotein (PML) nuclear bodies, splicing speckles and gems]²⁰⁻²⁵ (Fig. 1) are highly dynamic both internally and as discrete entities within the nucleus.^{13,26} Understanding in detail the functions of subnuclear structures and the mechanisms that regulate their occurrence is an active area of investigation.^{1,13,21,24-26}

Dynamics of the Nucleus

In order to avoid the potential for inappropriate rapid exchange of the constituent molecules, subnuclear structures must contend with structural and functional challenges that arise from the lack of boundary membranes (conceptually different from the cell cytoplasm where many key structures are membrane-limited).^{2,27-30} In this context a number of highly dynamic processes must be accommodated, including nuclear import and export across the NE,³¹ constant localized chromatin remodelling, local and long-range movements and interactions of chromosomes, movement of subnuclear structures within the nucleus and rearrangement of the NE.²⁹ The processes that lead to rearrangements of the NE and its components occur under a variety of physiological and pathological circumstances that occur during developmental, apoptotic and autophagic cell death pathways.

In recent years, a consensus model has been formulated that accounts for virtually all changes in NE structure and dynamics that occur during mitosis.³² In dividing metazoan cells, the interphase NE must undergo dramatic change. To successfully complete mitosis, the exclusively cytoplasmic microtubules of the spindle apparatus must contact the chromosomes normally enclosed within the nucleus and shielded by the NE. Mammalian cells disassemble their NE in prometaphase and undergo an ‘open mitosis’, effectively releasing the chromosomes into the cytoplasm allowing contact between the spindle apparatus and chromosome and facilitating their segregation following replication. The process of NE breakdown involves the disassembly and dispersal of all its structural units and their respective protein components (i.e., ONM, INM, NPCs and nuclear lamina). Once mitosis is completed, the dispersed NE components are reused to assemble new NE and nuclei in both mother and daughter cells. Functionally distinct classes of chromatin-interacting membrane proteins (e.g., lamin B, some nucleoporins) collaborate to rapidly re-establish the NE.³³ NE breakdown and re-assembly require the coordinated action of many cellular activities such as mitotic phosphorylation/dephosphorylation (e.g., cyclins and cyclin-dependent kinases), nucleocytoplasmic transport, the action of microtubule motor-proteins and membrane fusion.^{8,34} Note that clustering of NPCs and disintegration of the NE and lamina also occur as part of the cellular changes associated with apoptotic cell death in mammalian cells (characterized by chromatin condensation, the NE breakage and the DNA fragmentation).^{8,35}

In the yeast *S. cerevisiae*, by contrast, mitosis is ‘closed’ with the NE remaining intact. Nevertheless, the nucleus undergoes dramatic structural reorganization. Each mitotic spindle is confined

within the intact NE (NE during mitotic entry restricts the attachment of spindle microtubules to only chromosomes within that nucleus) and components (e.g., tubulin) required for the process are shuttled across the NE through NPCs.³⁶ The NE also remains intact during mating³⁷ and meiotic ascospore formation.³⁸

In addition to rearrangements of the nuclear membranes/nucleus during nuclear export/import, mitosis, mating and apoptosis accumulating evidence indicates the existence of a degradation process termed autophagy (specifically nucleophagy; see below), which is responsible for degradation of nuclear components in both yeast³⁹⁻⁴² and mammalian cells.⁴³ We now discuss the process of nucleophagy and its significance in cellular physiology and pathology, based mainly on the evidence from yeast studies and more recently from studies in mammalian systems.

Snapshot: Autophagy-Types, Mechanism(s), Function and Selectivity

Autophagy is a degradative process important for cellular homeostasis mediated via the vacuole (yeast cells) or the lysosome (mammalian cells).⁴⁴⁻⁵⁰ Three morphologically and mechanistically distinct modes of autophagy have been described: (1) chaperone-mediated autophagy (CMA) (described only in mammalian cells);^{46,51-53} (2) macroautophagy^{48,54} and (3) microautophagy.^{48,54} CMA targets cytosolic proteins bearing a KFERQ-like motif.⁵⁵ Whether there are proteins which shuttle between the cytosol and the nucleus that could undergo CMA during their cytosolic ‘residency’ has, to our knowledge, not been investigated. The latter two modes of autophagy have been described in both yeast and mammalian cells and can potentially serve as modes of nucleophagy.

Macroautophagy (often referred to simply as autophagy) is considered to be the major degradation route for turnover of cellular cytoplasmic constituents. Targets for autophagy include long-lived proteins, protein complexes (e.g., ribosomes) and aggregates, disease-related inclusions, exhausted, damaged or superfluous organelles (e.g., mitochondria, peroxisomes, ER), invading bacteria and viruses^{48,56-58} and most recently lipid droplets.^{59,60} During autophagy, portions of cytoplasm including macromolecules and whole organelles can be either non-selectively (bulk autophagy) or selectively (e.g., autophagic degradation of specific organelles such as mitochondria, peroxisomes) sequestered within double-membrane vesicles, termed autophagosomes (APs).^{48,54} Macroautophagy has been extensively characterized morphologically in both yeast and mammalian cells.^{49,61,62} Its mechanistic nature has been described, predominantly in yeast, as proceeding in seven sequential steps: (1) induction, (2) cargo selection and packaging, (3) AP nucleation, (4) AP expansion and completion, (5) retrieval of AP membrane components, (6) AP fusion with the vacuole (an example of point membrane fusion) and (7) breakdown of delivered cargo within the vacuole.^{46,48,49,54,63,64} The core (macro)autophagic machinery (autophagy-related *ATG* genes/*Atg* proteins) required for the process seems to be largely conserved in mammals^{65,66} and plants,^{66,67} as a number of homologues of yeast *ATG* genes have been identified.

Microautophagy (best described in yeast) is a related process with distinct membrane dynamics in which portions of cytoplasm

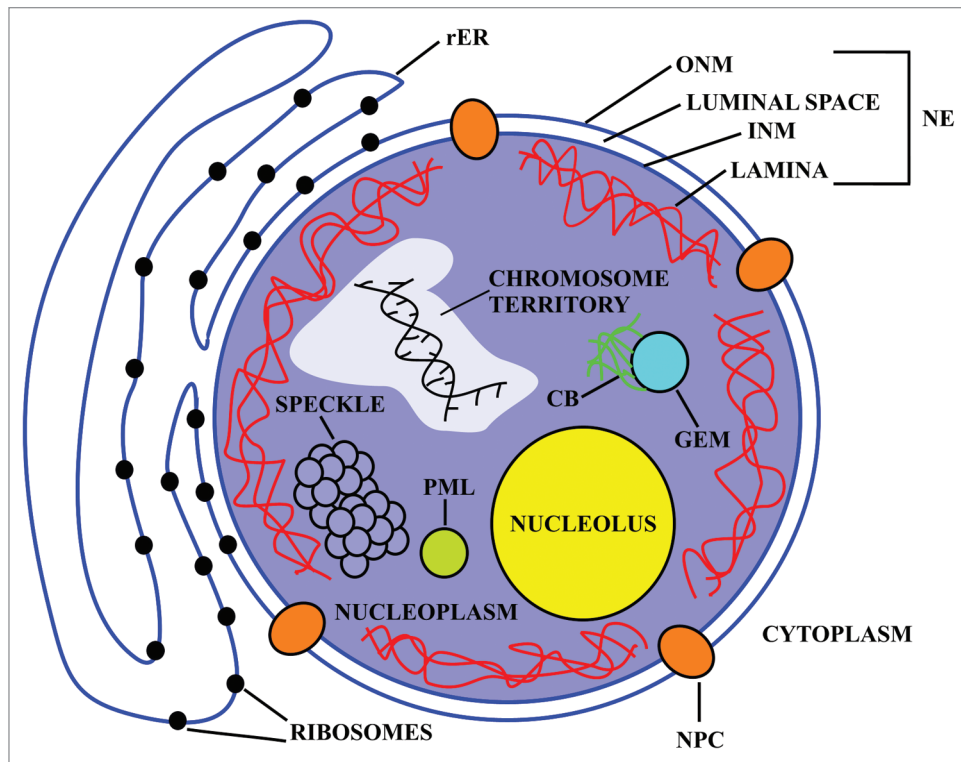


Figure 1. Schematic representation of a cell nucleus showing the main structural components. The nucleoplasm is enclosed by the nuclear envelope (NE), which contains the inner nuclear membrane (INM), luminal space and the outer nuclear membrane (ONM), the nuclear pore complexes (NPC), and the nuclear lamina. The ribosome-studded ONM is contiguous with the rough endoplasmic reticulum (rER), whereas the ribosome free INM faces the nucleoplasm and lamina. The ONM and INM are joined at the NPC. The yeast NE also contains NPCs and is continuous with the rER, but a functional counterpart of the metazoan lamina has not yet been described in yeast. This diagram also depicts some of the subnuclear structures located in the nucleoplasm, namely, nucleolus, chromosome territory, the Cajal body (CB), the promyelocytic leukemia oncoprotein (PML), splicing speckles and gems. Although each of these subnuclear structures represents an independent entity, remarkably, they are highly dynamic both internally and as discrete entities within the nucleus.^{13,26}

including organelles (data exists for mitochondria, peroxisomes and the nucleus) are sequestered by direct invagination of the vacuole membrane.^{39,42,68-70} Until recently, the components of the core *ATG* machinery involved in microautophagic processes were relatively poorly defined except for autophagic degradation of the peroxisomes (micropexophagy).^{68,69} Descriptions of microautophagic degradation of mitochondria (micromitophagy)⁷⁰ and of the nucleus (piecemeal microautophagy of the nucleus, PMN/micronucleophagy)^{39,42,71} have lead to the understanding that much of the core *ATG* machinery seems to be required for microautophagic processes.

Microautophagy was first described as a process in which the vacuolar membrane underwent localized invagination and ‘pinching off’ to form a vesicle within the vacuolar lumen. Components described as being required for this process, include some components of the homotypic vacuole fusion machinery and the vacuolar transporter chaperone-VTC complex (comprising Vtc1p-Vtc4p) which is present on the ER, vacuoles and at the cell periphery. On induction of autophagy by nutrient limitation the VTC complex is recruited to vacuoles becoming enriched on the membranes of autophagic tubes. Deletion of VTC genes blocks microautophagic uptake into vacuoles; although autophagic tubes still form the production of microautophagic vesicles from their tips is impaired.⁷²⁻⁷⁴

Additionally, the exit from rapamycin-induced growth (EGO) complex (composed of Ego1p, Gtr2p and Ego3p) has been implicated in the regulation of microautophagy.⁷⁵ The EGO signalling complex acts in conjunction with target of rapamycin (TOR) signalling complex, to positively regulate microautophagy following treatment of cells with rapamycin (a pharmacological compound which markedly upregulates autophagy). The internalisation of vacuolar membrane through microautophagy is considered to counterbalance the massive membrane influx toward the vacuolar membrane resulting from rapamycin-induced macroautophagy,⁷⁵ thereby maintaining vacuolar volume and membrane composition.⁷³ The requirement for the VTC, EGO and TOR complexes in organelle specific microautophagy in yeast and for microautophagy in general for mammalian cells remains to be determined.

Autophagic Degradation of the Nucleus by PMN: Requirements and Membrane Rearrangements

In *S. cerevisiae*, PMN leads to the pinching off and degradation of non-essential nuclear components including portions of the NE and the granular nucleolus enriched in pre-ribosomes, but excludes chromosomal DNA, NPCs and spindle pole bodies (SPBs).^{39,40,48,58,76} More experiments are required to definitively

NUCLEOPHAGY IN *SACCHAROMYCES CEREVISIAE*

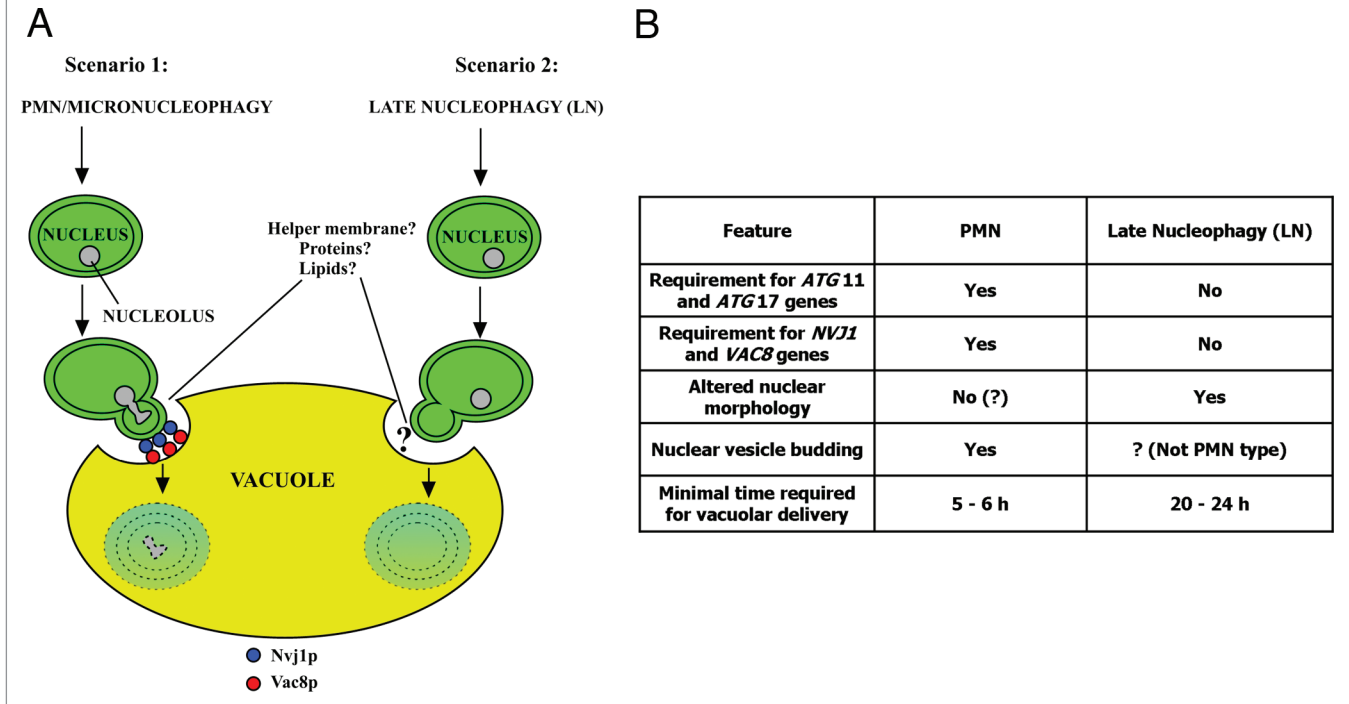


Figure 2. Mechanistic nature of nucleophagy in yeast. (A) Autophagy-induction by nitrogen starvation (early periods of nitrogen starvation) induces the formation of NV junctions involving Nvj1p and Vac8p which initiates PMN. Then the nucleus bulges into invaginations of the vacuolar membrane, followed by fission of a nuclear membrane vesicle, and its release into the vacuolar lumen, where it is finally degraded (Scenario 1). Upon prolonged periods of nitrogen starvation (>20 h) delivery of nucleoplasm occurs by late nucleophagy (LN) (Scenario 2). Furthermore, it is possible that during both PMN and late nucleophagy additional factors such as nuclear and vacuolar proteins and lipids may be involved in the regulation of respective processes. (B) Major differences between PMN (Scenario 1) and LN (Scenario 2) are indicated.

determine what components of the nucleus are degraded by PMN under various conditions. With regard to the mechanistic basis of PMN, Thumm and colleagues⁴² showed that efficient PMN requires much of the core *ATG* machinery. Required components are: (1) the two ubiquitin-like conjugation systems, coupling Atg12 to Atg5 and Atg8 to phosphatidylethanolamine (PE) respectively; (2) the Atg9 cycling system (except for Atg23 and Atg27); (3) phosphatidylinositol (PtdIns) 3-kinase complex; (4) macroautophagy specific proteins (Atg17, Atg29 and Atg31); and (5) some cytoplasm-to-vacuole targeting (Cvt)-specific proteins (Atg11 and partially Atg21 and Atg24). On this basis they concluded that PMN represents a “true microautophagic process,” which can also be termed micronucleophagy (Scenario 1; Fig. 2A). Atg8p has been demonstrated to be involved in tethering between adjacent membranes and stimulating membrane hemifusion in vitro.^{77,78} Such functions, which mimic expansion of the autophagosomal membrane during macroautophagy, may contribute to membrane growth during microautophagy.

PMN occurs in the context of nucleus-vacuole (NV) junctions between the nucleus and the vacuole generated by the interaction between two key proteins, a vacuolar membrane protein, Vac8p, and the ONM protein Nvj1p (Scenario 1; Fig. 2A).^{40,79} Upon starvation, these NV junctions bulge into invaginations of the vacuole, after which a portion of the nucleus (“micronucleus”) buds off and after fusion of the vacuolar

extensions, a (PMN) vesicle limited by three membranes (the outermost vacuolar membrane, the two nuclear membrane; ONM and INM) is released into the vacuole and subsequently degraded (Scenario 1; Fig. 2A).^{39,40,42,58} Interestingly the vesicles produced in PMN can be very large, seemingly almost as large as the nucleus. It is not clear why these vesicles need to be of such size, or whether size is determined by the specific cargo being delivered to the vacuole. It has been reported that in addition to the presence of Vac8p and Nvj1p at NV junction sites, and the requirement of the core autophagic machinery (see above), lipid homeostasis (see below) is important also for the biogenesis of PMN vesicles.^{40,42,80,81} Thus, yeast mutants lacking certain proteins involved in lipid homeostasis exhibit defects in the maintenance of NE structure, accumulation of PMN intermediates, reduced size of PMN structures, perturbed intracellular sterol-lipid distributions and disrupted vacuole structure. However, a direct link between the factors involved in sterol-lipid homeostasis and the molecular mechanism of PMN has yet to be established.⁴⁰

In addition to its involvement in the formation of NV junctions, Vac8p is a multi-purpose adaptor protein required for several other vacuolar processes in yeast such as the Cvt pathway, homotypic vacuole-vacuole fusion and the transfer and inheritance of vacuoles to budding daughter cells.⁸²⁻⁸⁵ In addition to Nvj1, other binding partners of Vac8p have been

identified by yeast two hybrid analysis and include: Vac17p (a component of the actin-based vacuole inheritance apparatus),⁸⁶ Tco89p (a vacuole membrane protein reported to be a member of the yeast TOR complex 1 and required for maintenance of cell wall integrity),⁸⁷ Atg13p (a phosphorylated protein required for Cvt and autophagy),⁸⁴ Vid21p (a component of the conserved NuA4 histone acetyltransferase complex),⁸⁸ Vab2p (a vacuolar membrane protein that interacts with the vacuolar ATPase subunit Vma8p),⁷⁹ Tao3p (involved in cell morphogenesis and proliferation)⁸⁹ and two uncharacterized proteins Yel043w and Yfr035c.⁸⁵ However, whether these Vac8p binding partners function in PMN has yet to be elucidated.

In addition to mediating PMN, Nvj1p also functions in sequestering two conserved proteins, namely, Osh1p and Tsc13p,^{40,90,91} with roles in lipid biosynthesis and trafficking to the NV junctions. Osh1p is thought to mediate sterol-dependent trafficking of the high-affinity tryptophan permease, Tat2p, that is routed to either the plasma membrane or the vacuole depending on the concentration of tryptophan in the medium.^{90,92} Targeting of Osh1p to NV junctions is specified by the ankyrin repeat region; Osh1p is diffusely distributed in strains lacking either Nvj1p or Vac8p alone, implying that binding requires both Nvj1p and Vac8p. Targeting would seem to require correct formation of the NV junction structure, such that Osh1p binds only to the Nvj1p/Vac8p complex, or to some other factor recruited to this complex.⁹³ Interestingly, the human homologue of Osh1p, *ORP1L*, localises to perinuclear lysosomes and autophagic vacuoles (AVs) when overexpressed in HeLa cells,^{40,94} but the relationship between its localization and any function in mammalian nucleophagy is yet to be fully investigated.

The enoyl-CoA reductase Tsc13p is an essential ER membrane protein that catalyses the terminal step of very-long-chain fatty acid (VLCFA) biosynthesis, but which is also highly enriched in NV junctions.^{40,90,91,95,96} Whether Tsc13p requires Nvj1p or Vac8p for localization to NV junctions remains to be determined. VLCFAs are important constituents of several complex lipid species in yeast including ceramides, sphingolipids, inositolphospholipids and glycosylphosphatidylinositol anchors, and as such, play important roles in membrane and lipid raft biogenesis, membrane fluidity and thickness and cell signalling.^{40,90,91} The human homologue of Tsc13p, *SC2*, exhibits similar enoyl-CoA reductase activity during fatty acid elongation *in vitro*.^{40,97}

Vesicles, highly enriched in Tsc13p, are observed to bud off the NE into the lumen of the vacuole (these vesicles markedly resemble PMN vesicles) when cells enter stationary phase in the presence of non-fermentable carbon sources, or in *elo2* and *elo3* mutants (both genes are required for VLCFA synthesis) indicating that nuclear-vacuolar interactions may provide a preferred site for NE recycling.⁹⁵ In this context, one could ask what might be the physiological role of the dual localization of Tsc13p at ER and NV junctions, or of other components of the fatty acid elongation system which might be found at the sites of nuclear-vacuolar interaction.⁹⁵

Why micronucleophagy requires such a complex apparatus to achieve membrane fusion between the nucleus and vacuole has

been considered by Thumm and colleagues.⁴² They argue that fusion of the vacuolar and nuclear membranes at a site of invagination is unlikely to be able to occur at a single point based on observations of homotypic fusion of vacuoles. Vacuolar fusion does not take place at a single fusion point, which then expands radially, but at multiple vertex sites (microdomains at the apposed membranes).⁹⁸ Presumably, several SNARE-complexes must form simultaneously over a larger surface area to generate enough force to achieve fusion. Krick et al.⁴² speculated that this problem could be overcome by using a membranous helper structure similar to that observed in micropexophagy in *Pichia pastoris*. Here, a cluster of peroxisomes is enclosed by vacuolar membrane protrusions and/or segmented vacuoles in concert with a newly formed membrane structure, the micropexophagy-specific membrane apparatus (MIPA), which mediates the enclosure of the vacuolar membrane,^{68,99} by heterotypical fusion with the surrounding vacuolar membrane (Scenario 1; Fig. 2A). At present there is no evidence to support the involvement of a MIPA-like apparatus in nucleophagy.

A Second Form of Autophagic Degradation of the Yeast Nucleus

We have recently obtained evidence for a second form of nucleophagy—late nucleophagy (LN)—in yeast that can be detected after prolonged periods of nitrogen starvation (Mijaljica et al., manuscript in preparation). Dual labelling of cells with Nvj1p-EYFP (an outer NE reporter of PMN)³⁹ and NAB35-DsRed.T3 (nucleoplasm reporter) demonstrated that whereas induction of PMN can be detected as early as 3 hours of nitrogen starvation as reported previously,³⁹ LN can be detected only after 20 hours. In addition to this clear temporal distinction between the two processes, our data suggest that they are also spatially separated, as we rarely observe dual-labelling of nuclear material (vesicles) taken up into the vacuole. LN does not require the same components of the core macroautophagic machinery, or even Vac8p or Nvj1, essential components of NV junctions in PMN. The inhibition of LN in some *atg* mutants is accompanied by gross alterations in the nuclear morphology (Scenario 2; Fig. 2A), again emphasising the differences between this form of nucleophagy and PMN. The differences between PMN and LN have been summarised in Figure 2B.

It is not clear (yet) whether these alterations are a consequence of LN being mechanistically deranged (and therefore the inability of the cell to remove material such as damaged components from the nucleus), or whether the conditions of cell growth (nitrogen starvation) under which the phenotype is viewed otherwise leads to the depletion or mis-localization of a key enzymatic activity in relation to maintenance of membrane morphology.

Previous findings have established that the amount and types of phospholipids are crucial for the biogenesis and homeostasis of the NE. Dramatic effects on yeast nuclear morphology have been reported in yeast strains perturbed in phospholipid homeostasis due to deletion of selected genes. For example, in cells null for expression of each of *NEMI* or *PAH1* display irregularly shaped nuclei.^{18,100} A striking nuclear membrane phenotype has

Table 1. Autophagic degradation of the nucleus and its components in yeast and mammalian cells

| Nucleus/Nuclear component | Evidence for autophagic process(s) involved in degradation | |
|------------------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| | Yeast cells | Mammalian cells |
| Entire nucleus | Not determined | Mechanistically uncharacterized nucleophagy ¹⁰³ |
| Nucleoplasm | Nucleophagy ^{41,126,127} PMN ⁴² | Not determined |
| Inner Nuclear Membrane (INM) | PMN ³⁹ | INM and ONM participate in the formation of autophagosome-like membranes during viral infection of macrophages ^{123,124} |
| Outer Nuclear Membrane (ONM) | PMN ³⁹ | |
| Nuclear Pore Complex (NPC) | Not degraded ^{39,40} | Not determined |
| Nucleolus | PMN ³⁹ | Not determined |
| Nuclear Lamina | Yeast cells lack nuclear lamins | Mechanistically uncharacterized nucleophagy ⁴³ |
| Chromosomes/Chromosomal DNA | Not degraded ^{39,40} | Mechanistically uncharacterized nucleophagy ^{43,104} |

been reported in cells of a temperature-conditional *mtr7* mutant, defective in acetyl-CoA carboxylase, linking VLCFA synthesis to the structure and function of the nuclear membrane-pore complex.¹⁰¹

It is possible that alterations in nuclear morphology observed in cells null for autophagy genes required for efficient LN may relate to disturbance of phospholipid and/or VLCFA homeostasis. A change in lipid composition and distribution in the nuclear membranes may be a signal for nucleophagy events.¹⁰²

Autophagic Degradation of the Nucleus and its Components in Mammalian Cells

The role of autophagy in nuclear turnover of mammalian cells has recently received increased attention. Early observations were disparate in nature and provided few details with the respect to the type and role of the particular autophagic processes described (Table 1). The degradation of the entire nucleus was reported to occur in cultured murine seminal vesicle epithelial cells through an autophagic process termed nucleophagy, but without any description of its mechanism (i.e., mechanistically uncharacterized nucleophagy).¹⁰³ More recently it was reported that sequestration of mitotic (M-phase) chromosomes in APs of Chang liver cells could be induced by an OH[•] burst.¹⁰⁴ These observations raised the intriguing question of how the normal mitotic spindle and chromosomes that are transiently resident in the cytosol compared to their normal sequestration within the NE during interphase, are protected from persistently robust autophagy that occurs during mitosis.¹⁰⁵ A third study demonstrated that senescent keratinocytes seem to die through massive and specific autophagic degradation of their nuclei and mitochondria, although degradation of other cellular organelles was not examined.¹⁰⁶ Other findings supported the role of autophagy in the death of senescent keratinocytes. The autophagy inhibitor 3-methyladenine can delay the death of senescent cells. Moreover, if the acidification of lysosomes, required for the final degradation of cell components, was blocked then AVs full of debris accumulated inside corpses. Finally, dying senescent cells acquired a particular intracellular organization, whereby a cyokeratin network developed and partitioned the cell into a cortical domain devoid of organelles and a central domain containing a high number of

AVs, most of the mitochondria, and the nucleus. These observations gave rise to the assumption that due to their close proximity to AVs, nuclei and mitochondria could be degraded within such AVs. This notion was supported by the altered morphology of the nuclei and mitochondria within the central domain and by the level of DNA degradation observed.¹⁰⁶

It is possible that the nuclei of senescent cells in general could be targeted for autophagy simply because their DNA is damaged, since inhibition of DNA-protein kinase, a nuclear kinase involved in DNA-break signaling, sensitizes to autophagy in malignant glioma cells,¹⁰⁷ suggesting that persistence of damaged DNA can activate the autophagic process. Similarly, both an increase in Beclin-1 (mammalian homologue of Atg6p) expression and a concomitant increase in autophagic activity occur following treatment with the DNA-damaging agent etoposide.¹⁰⁸ Furthermore, DNA damage has been shown to accumulate in cancer cells deficient in autophagy.¹⁰⁹

Accumulating evidence suggests that reduced autophagy promotes DNA damage, gene amplification, chromosome instability, and aneuploidy, all of which are clinically associated with tumor progression and poor prognosis. Similar findings with allelic loss of *beclin1* in immortalized breast epithelial cells and immortalized, autophagy-defective *atg5*^{-/-} mouse embryo fibroblasts (MEFs) suggest that these observations are independent of tissue type. Analogous to the mammalian central nervous system where targeted deletion of either *atg5* or *atg7* revealed the protective role of constitutive autophagy through prevention of the accumulation of polyubiquitinated proteins and neurodegeneration, in tumor cells, preventing the genome damage through autophagy may be an essential cell-autonomous mechanism for tumor suppression.¹¹⁰ Furthermore, autophagy—defective *beclin1*^{-/-} immortalized baby mouse kidney epithelial (iBMK) cells expressing anti-apoptotic Bcl-2 showed profound microtubule and centrosome abnormalities, including heterogeneity in cell and nuclear size and shape and an increase in the percentage of cells with centrosome abnormalities including increased centrosome number. Abnormalities in the microtubule framework can arise due to abnormally large cell size. Moreover, supernumerary centrosomes and large nuclei are defining traits of excess DNA content as well as genomic instability. These abnormalities in *beclin1*^{-/-} iBMK cells suggested that autophagy and maintenance of metabolism

might be crucial for limiting DNA damage and maintaining genome integrity.¹¹⁰

Most recently, it has been demonstrated that perinuclear APs/autolysosomes (sometimes bigger than the nucleus itself) contain extruded nuclear components in nuclear envelopathies/laminopathies caused by mutations in the genes encoding A-type lamins (*LMNA*) or emerin (*EMD*). These APs and autolysosomes were decorated with autophagy-related proteins (e.g., LC3) and the lysosomal marker protein, LAMP2. Moreover, APs appeared to contain the damaged portions of nuclei as demonstrated by γ H2AX (a marker of DNA double-strand breaks) immunostaining.⁴³

Nucleophagy: In Physiology and Pathophysiology

Focusing on *S. cerevisiae*. In unicellular organisms, the primary role of autophagic processes (in general) is to regulate intracellular homeostasis (e.g., in response to starvation). Thus, PMN could function in yeast as a quality control mechanism (regulation of nuclear volume, quantity and quality) to dispose of damaged nuclear components or bulky nuclear aggregates that are resistant to attack by the proteasome.^{39,40} Strong evidence now indicates that proteasomes are active in both the cytoplasm (the ubiquitin-proteasome system-UPS) and the cell nucleus (nUPS).¹¹¹ nUPS is active both in the nucleoplasm and distinct subnuclear structures (e.g., PML nuclear bodies, nuclear speckles) where it plays a major role in controlling the initial steps of gene expression and nuclear quality-control mechanisms including DNA repair.^{111,112} In mammalian cells, specific inhibition of proteasomal degradation by inhibitors such as lactacystin stabilises nuclear proteins such as histone H2A and PML protein. However, these proteins accumulate in the subnuclear structures in which they normally reside upon inhibition of proteasomes, findings that suggest that they represent substrates of the nUPS. By contrast, nucleolar and NE proteins such as fibrillarin and lamin A/C do not appear to represent proteasome substrates under normal conditions.^{111,112} In yeast cells, both the UPS and nUPS appear to play a more restricted role. Limitations in the use of proteasomal inhibitors (because only β -lactone can penetrate cells¹¹³), make it difficult to test if inhibition of nUPS enhances the level of PMN or late nucleophagy.

Focusing on mammals. In higher eukaryotes autophagy is involved in a wide range of physiological and pathological processes, including stress responses, development, cell differentiation, antigen presentation, cancer, aging, neurodegeneration (e.g., Parkinson and Alzheimer diseases), various myopathies (e.g., Danon disease) and cell death.^{48,50,56,57,64,114,115} Recently, it has become evident that in several pathological situations where autophagy has a beneficial role, this degradation pathway is able to specifically and/or selectively eliminate unwanted structures including cellular organelles or bacterial and viral pathogens.^{64,116}

It could be argued that autophagic degradation of the nucleus in mammalian cells is not generally necessary because, unlike yeast cells which have a closed nucleus during mitosis, they could utilise 'standard' autophagic degradation processes (either macro- or microautophagic) during S phase when the NE has been

dis-assembled. As suggested by Kvam and Goldfarb⁴⁰ specialized autophagy for turnover of the nucleus may be more important for long-lived amitotic cells such as neurons. Micronuclei are found in a variety of mammalian cells¹¹⁷ for example, micronuclei have been found in the cytoplasm of Bloom syndrome cells during S phase.¹¹⁸ While it is beyond the scope of this review to consider the production and fate of micronuclei, it is presently unknown whether micronuclei are turned over by autophagy. This could occur by a microautophagic process through the direct interaction of micronuclei membranes with lysosomes, or micronuclei could be sequestered in APs by non-selective macroautophagy. In both cases these processes could be considered as nucleophagy.

Links between human pathology and possible autophagy of the nucleus are now becoming apparent. Approximately 25% of cases of Diamond Blackfan Anemia (DBA), a severe hypoplastic anaemia, are linked to heterozygous mutations in the gene encoding ribosomal protein S19 and a haploinsufficiency for this protein.¹¹⁹ When amino acid substitutions from DBA patients known to induce defects in the processing of the pre-rRNA are introduced into yeast Rps19p, defects similar to those observed in cells under-expressing Rps19p are observed. A conspicuous feature in a majority of Rps19p-depleted yeast cells, as observed in electron microscopy sections, was what appeared to be the vacuole engulfing the nucleolus.¹²⁰ These results demonstrate the effect of DBA-associated mutations on the function of Rps19p, strongly connecting the pathology to ribosome biogenesis and indicate a role for autophagy in the pathogenesis of disease.

The mutation of p.H222P in *LMNA* is one of several mutations causing muscular dystrophy in humans. A homozygous knock-in mouse model carrying the mutation, *Lmna*^{H222P/H222P} reproduces the phenotype of human muscular dystrophy due to *LMNA* mutations.¹²¹ Recently, Nishino and colleagues⁴³ demonstrated, using *Lmna*^{H222P/H222P} MEFs, that where the NE interfaces with APs/autolysosomes there was an increase of NE proteins. The autophagic nature of such processes was further supported by the finding that LC3-II (a marker of autophagy that decorates APs) is upregulated in these cells. Furthermore, inhibition of autophagy in these cells led to the accumulation of nuclear abnormalities (irregular shape of the nucleus, the disruption of nuclear membrane) and reduced cell viability, strongly suggesting a beneficial role of nucleophagy. Interestingly evidence for the occurrence of nucleophagy was observed even in a small percentage of wild-type cells, suggesting that autophagic degradation of nuclear components is not confined to the disease condition. It was further suggested that autophagic degradation of particular nuclear components could contribute to the rapid repair of the nuclear membrane and prevention of cell death. However, the precise role of nucleophagy in *Lmna*^{H222P/H222P} MEFs, remains to be fully elucidated.⁴³

An intriguing observation suggests the NE and its interrelationship with an autophagic process may be crucial to the course of viral infection. In herpes simplex virus type 1 (HSV-1) infected macrophages, four-layered membrane structures (comprising the double AP membrane and NE membrane) were seen to emerge from the NE, disconnect from the nucleus and accumulate in the cytoplasm as a late response to infection (around 8 hours after

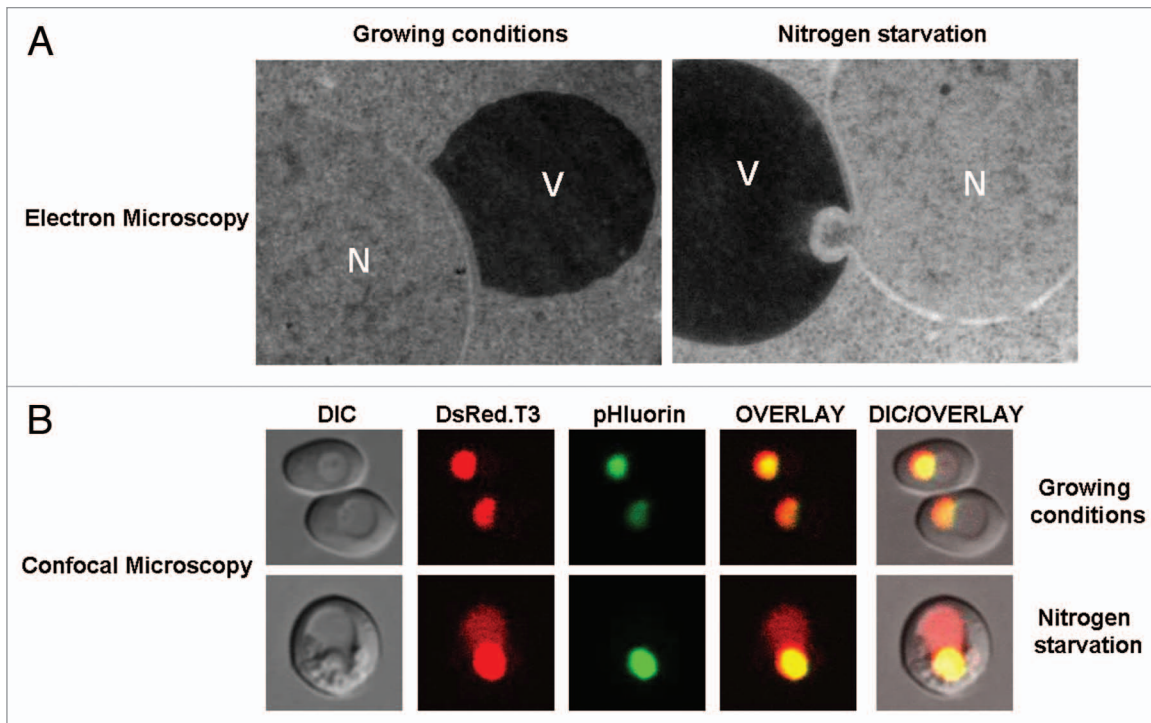


Figure 3. Monitoring autophagic degradation of the nucleus in yeast. (A) Electron micrograph showing the contact between the outer membrane of the nucleus and the vacuolar membrane under both growing and nitrogen starvation conditions (24 h starvation). Note the partitioning of the nucleus (N) into the vacuole (V) under nitrogen starvation conditions where both the INM and ONM exvaginate in tandem into the vacuole (this could represent either a PMN or LN event). (B) Live-cell confocal imaging of delivery of the nucleus in the vacuole (LN) using NAB35-Rosella biosensor.^{41,126,127} Cells were either grown in the presence of both carbon and nitrogen (growing conditions) or subjected to nitrogen starvation for 24 h. This fluorescent reporter is targeted to the nucleoplasm, allowing nucleophagy to be monitored during the phase that represents uptake of material into the yeast vacuole for degradation. The reporter is a dual colour emission biosensor comprising a relatively pH-stable red fluorescent protein variant, DsRed.T3 and a pH-sensitive green fluorescent protein variant, pHluorin.^{126,127} NAB35-Rosella exhibits both red and green fluorescence in the nucleus (neutral pH environment) (growing conditions and nitrogen starvation), but loses green fluorescence and retains red fluorescence when within the vacuole (nitrogen starvation).

infection). These four-layered structures were decorated with LC3 and subsequently fused with lysosomes. It seems that during this process, the nucleus is the source of the membrane that becomes the autophagosomal membrane. Thus the potential advantage of assembly of viral particles in the protected environment of the nucleus prior to movement through the NE, is counteracted by the nucleophagic response.^{122,123}

Unanswered Questions

Yeast. Many questions with regards to micronucleophagy remain unanswered. For example, what additional proteins (of either nuclear or vacuolar membranes) are required for establishing the exceptionally tight associations between Nvj1p and Vac8p? What is the influence of lipid composition of the respective membranes? Do the sites of vesicle formation represent sites used once, or for multiple sequential events? Do NV junctions form at specific (as yet unidentified) sub-domains of the nuclear and/or vacuolar membranes and what controls inclusion/exclusion of various proteins and/or lipids at those NV junctions? In this context it has been reported that NV junctions form at regions lacking NPCs.^{39,102} Another intriguing question is which nuclear components (target substrates) can be degraded

by PMN and/or LN and whether the same nuclear components are degraded under different autophagy-induced conditions (e.g., nitrogen and/or carbon starvation, rapamycin treatment). Also, it is not clear if there is a temporal difference in the recruitment of different components of the nucleus into PMN vesicles or during LN.

Mammalian cells. The findings of Park et al.⁴³ indicate that the giant APs/autolysosomes observed appear to degrade the extruded nuclear components. Although such giant APs are quite unusual, there is precedent for large APs in mammalian cells. First the observations of Kovács et al.¹⁰³ referred to above and second the APs reported to sequester infecting group A streptococcus in HeLa cells.^{124,125} The form of nucleophagy being observed in all these examples would appear to be macroautophagy. The involvement of microautophagic processes cannot yet be unequivocally ruled out. It may be that the particular conditions applying in cells influence the form of nucleophagy induced. Thus, in yeast PMN and LN are induced by nitrogen depletion or rapamycin induction, whereas in *Lmna*^{H222P/H222P} cells it appears the NE structural defect relating to defective lamina structure contributes to the molecular mechanism. Interestingly, Park et al.⁴³ noted evidence of nucleophagy in (apparently) wild-type cells, but at much lower frequency, implying that nucleophagy

can occur as a result of other conditions that promote nuclear damage. Understanding what these conditions might be, the nature of the damage sustained when these conditions persist and the identity of the nuclear components subsequently degraded by nucleophagy, await elucidation.

Concluding Comments and Prospectus

The role of autophagy in the degradation of nuclear components is gaining wider significance, with reports of nucleophagy in mammalian cells, now building upon the foundation of observations in yeast. Even though there remains much to be determined regarding the mechanistic nature of the processes described to date, it is clear that nucleophagy involves largely yet to be revealed intricacies of membrane dynamics. The notion of pinching off parts (“pieces”) of the nucleus and eliminating them by nucleophagy (Fig. 3), in order to maintain the vitality of the nucleus and the cell is an attractive proposition. Presumably, nucleophagy (in whatever form) is sufficient to ‘repair’ damaged nuclei so that cells can maintain normal

function and avoid cell death by apoptosis. Whether a similar mechanism apply across species (i.e., conservation across evolution) and under various environmental cues, has yet to be investigated in detail and awaits further studies in mammalian cells in particular, and may well be informed by studies in other genetically tractable metazoan species such as *Drosophila melanogaster*, *Caenorhabditis elegans* and *Danio rerio* (zebrafish). Thus, the next few years hold the promise of significant progress in our understanding of the mechanisms of nucleophagy and the accompanying intricacies of nuclear membrane rearrangements.

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