Nucleophagy at a glance

Dalibor Mijaljica*,‡ and Rodney J. Devenish‡

Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton campus, VIC 3800, Australia

*Present address: Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, 399 Royal Parade, Parkville, VIC 3052, Australia

‡Authors for correspondence (Dalibor.Mijaljica@monash.edu; Rodney.Devenish@monash.edu)

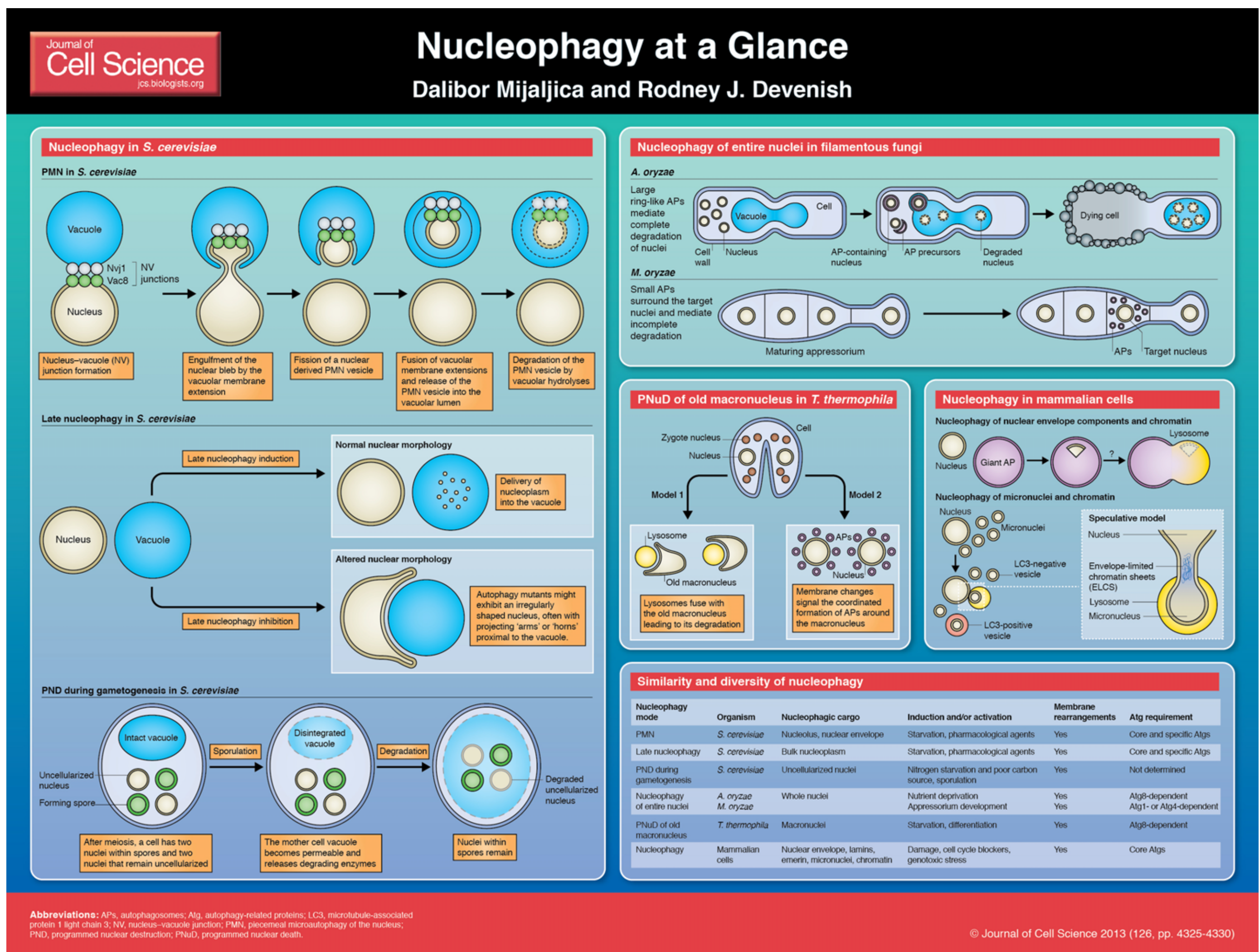
Journal of Cell Science 126, 4325–4330
© 2013. Published by The Company of Biologists Ltd
doi: 10.1242/jcs.133090

Summary

Under certain circumstances, the removal of damaged or non-essential parts of the nucleus, or even an entire nucleus, is crucial in order to promote cell longevity and enable proper function. A selective form of autophagy, known as nucleophagy, can be used to accomplish the degradation of nucleus-derived material. In this Cell Science at a Glance article and the accompanying poster, we summarize the similarities and differences between the divergent modes of nucleophagy that have been described to date, emphasizing, where possible, the molecular mechanism, the membrane interactions and rearrangements, and the nature of the nucleus-derived material that is degraded. In turn, we will consider nucleophagy processes in the lower eukaryotes, the budding yeast *Saccharomyces cerevisiae*, filamentous fungi *Aspergillus* and *Magnaporthe oryzae* and the ciliated protozoan *Tetrahymena thermophila*, and finally in mammalian cells. We will also briefly discuss the emerging links between nucleophagy and human disease.

Introduction

The cell nucleus is a cellular organelle whose *raison d'etre* is the maintenance and expression of the genome. Maintenance of nuclear structure and organization is essential for the vitality of most cell types (Mijaljica et al., 2010). Therefore, just as for other cellular organelles, we would anticipate 'surveillance' by processes that act to minimize nuclear damage by removal of damaged or non-functional components. Studies in both yeast and mammalian cells support the contention that selective digestion of portions of the nucleus can occur in order to maintain nuclear integrity (Roberts et al., 2003; Park et al., 2009; Mijaljica et al., 2012). By contrast, in other systems, such as



Nucleophagy mode	Organism	Nucleophagic cargo	Induction and/or activation	Membrane rearrangements	Atg requirement
PMN	*S. cerevisiae*	Nucleolus, nuclear envelope	Starvation, pharmacological agents	Yes	Core and specific Atgs
Late nucleophagy	*S. cerevisiae*	Bulk nucleoplasm	Starvation, pharmacological agents	Yes	Core and specific Atgs
PND during gametogenesis	*S. cerevisiae*	Uncellularized nuclei	Nitrogen starvation and poor carbon source, sporulation	Yes	Not determined
Nucleophagy of entire nuclei	*A. oryzae* *M. oryzae*	Whole nuclei	Nutrient deprivation Appressorium development	Yes Yes	Atg8-dependent Atg1- or Atg4-dependent
PNuD of old macronucleus	*T. thermophila*	Macronuclei	Starvation, differentiation	Yes	Atg8-dependent
Nucleophagy	Mammalian cells	Nuclear envelope, lamins, emerin, micronuclei, chromatin	Damage, cell cycle blockers, genotoxic stress	Yes	Core Atgs

(See poster insert)

the ciliated protozoan *Tetrahymena thermophila* and some filamentous fungi, the evidence suggests that selective digestion of whole nuclei occurs as part of a program of cellular development or mechanism of infection (Akematsu et al., 2010; Shoji et al., 2010; He et al., 2012). Autophagy is a homeostatic intracellular degradation system widely conserved in eukaryotes that is known to operate in a selective manner to remove organelles, such as mitochondria or peroxisomes, and which, as will be discussed below, can accomplish the degradation of nucleus-derived cargo or whole nuclei. Autophagy-related genes (the Atgs), which encode proteins comprising the core autophagic machinery, regulate all types of autophagy (Geng et al., 2013).

Nucleophagy as a selective form of autophagy

Nucleophagy is the selective removal of nuclear material from a cell by autophagy; it can occur either as a selective mode of macroautophagy or microautophagy, being referred to as macronucleophagy or micronucleophagy, respectively. In macronucleophagy, autophagosomes sequester the nucleus-derived cargo and subsequently fuse with the vacuole or lysosomes, which results in the degradation of their contents (Park et al., 2009; Liu and Yao, 2012). By contrast, micronucleophagy involves the direct engulfment of the nucleus-derived cargo by invagination, protrusion and/or septation of the vacuolar or lysosomal limiting membrane (Kvam and Goldfarb, 2007; Krick et al., 2008; Krick et al., 2009).

Nucleophagy in lower eukaryotes

A number of divergent modes of nucleophagy exist and they can be differentiated principally by the mechanisms that mediate the delivery of the nucleus-derived cargo to vacuole or lysosomes, as well as by the nature of the cargo. Below, we discuss those nucleophagy processes for which some mechanistic information is available.

Piecemeal microautophagy of the nucleus

Piecemeal microautophagy of the nucleus (PMN) progresses in five distinct steps (see Poster) in order to remove and degrade small blebs of the *Saccharomyces cerevisiae* nucleus. It is initiated at nucleus–vacuole (NV) junctions, highly localized membrane–membrane contact zones at which membrane reorganization occurs (Kvam and Goldfarb, 2006; Krick et al., 2009; Dawaliby and Mayer, 2010). NV junctions are formed by interactions between Vac8, located in the vacuolar membrane, and Nvj1, in the nuclear envelope (step 1). Conditions that normally induce autophagy (e.g. a short-duration nitrogen starvation) result in the invagination of membranes that are associated with NV junctions into the vacuolar lumen. These invaginations consist of three membranes (the surrounding vacuolar membrane, which will eventually encompass the nuclear bleb that is comprised of both nuclear outer and inner membranes) and a portion of underlying nucleoplasm (step 2). Sequestration of the nuclear cargo occurs by fission of both nuclear membranes and fusion of the vacuolar membrane extensions (step 3). With the fusion of the vacuolar membrane extensions, the triple-membrane PMN vesicle is released into the vacuolar lumen (step 4), where it is eventually degraded by the resident vacuolar hydrolases (step 5) (Roberts et al., 2003; Kvam and Goldfarb, 2007; Krick et al., 2009). PMN is classified as microautophagy because the delivery of the nuclear bleb to the vacuole occurs by direct invagination and scission of the vacuole membrane, without involvement of autophagosome structures. Notably, components of the homotypic vacuole fusion machinery that are required for macroautophagy processes are not needed, emphasizing that PMN is a microautophagy process (Krick et al., 2008; Krick et al., 2009; Millen et al., 2009). As yeast has a closed mitosis (i.e. the replicated chromosomes divide within an intact nucleus), PMN provides a mechanism by which potential nuclear cargo destined for vacuolar degradation that would otherwise be enclosed – and rendered inaccessible – by the nuclear envelope can be accessed. The cargo that is sequestered by PMN includes non-essential nuclear components, such as the granular nucleolus that is enriched in pre-ribosomes, as well as the surrounding portion of nuclear membranes (Roberts et al., 2003). Nucleus-derived components, such as bulk chromatin, nuclear pore complexes and spindle pole bodies are excluded from PMN vesicles (Roberts et al., 2003; Krick et al., 2009; Millen et al., 2009).

Efficient PMN requires the core Atg genes and a set of subtype-specific Atg genes. The dependence of PMN on Atg genes seems to be restricted to the final step where the vacuolar membrane that surrounds the pinched-off piece of nucleus fuses to 'close' the outermost membrane (vacuolar-derived) of the PMN vesicle. In cells deleted for individual Atg genes, NV junctions and blebs still form (Krick et al., 2008; Krick et al., 2009).

Late nucleophagy

Late nucleophagy (see Poster) of bulk nucleoplasm in *S. cerevisiae* can be detected only after prolonged periods (20–24 hours) of nitrogen starvation (Mijaljica et al., 2010; Mijaljica et al., 2012). In addition to this clear temporal difference between PMN and late nucleophagy, these two processes are also spatially separated (Mijaljica et al., 2012). Late nucleophagy is also classified as a microautophagy process because delivery of the nuclear cargo occurs without involvement of autophagosome structures. In contrast to PMN, late nucleophagy can occur in the absence of Nvj1 or Vac8, and, therefore, the formation of NV junctions. Although some components of the core Atg machinery are required for late nucleophagy, the macroautophagy-specific Atg11 is not. Intriguingly, late nucleophagy also does not require the components of the Vps34-containing PtdIns(3)*P*-kinase complex I (Mijaljica et al., 2012), although the mechanistic significance of this is yet to be established. Interestingly, inhibition of late nucleophagy in some autophagy-deficient mutants is accompanied by morphological alterations of the nucleus (Mijaljica et al., 2012). However, it is not clear whether these alterations are a consequence of misregulated late nucleophagy, or whether the conditions of cell growth, such as prolonged nitrogen starvation under which this phenotype is observed, leads to the depletion or mislocalization of one or more key factors (i.e. proteins or lipid) that are important for maintaining nuclear membrane morphology. At present there is no evidence that different types of cargo are delivered to the vacuole during late nucleophagy. It is possible that PMN and late nucleophagy facilitate the

sequestration of different nuclear cargoes, but this remains to be established.

Programmed nuclear destruction during gametogenesis in *S. cerevisiae*

Diploid cells that are grown on a non-fermentable carbon source will normally enter meiosis in response to nitrogen starvation to produce four haploid nuclei, which are then packaged into spores. Under conditions of suboptimal carbon supply, cells produce only two spores and the two nuclei that do not form spores remain uncellularized in a common cytoplasm. Following spore formation, the mother cell vacuole, which is not partitioned into the daughter spores after meiosis, undergoes the final steps of disintegration. The release of the lytic contents of the vacuole into the cytoplasm leads to the destruction of the two uncellularized nuclei (see Poster) (Eastwood et al., 2012; Eastwood et al., 2013). This type of lytic dissolution of cellular contents including nuclei does not appear to be a unique feature of yeast programmed nuclear destruction (PND), as it is reminiscent of 'mega-autophagy', the rupture of the central vacuole in plant cells as part of programmed cell death (van Doorn and Woltering, 2005; van Doorn and Woltering, 2010). That mega-autophagy is not the conventional form of macroautophagy is emphasized by observations that show typical autophagosome structures are not formed to sequester the uncellularized nuclei (Eastwood et al., 2012; Eastwood et al., 2013).

Macroautophagy-mediated degradation of entire nuclei in filamentous fungi

In the filamentous fungus *Aspergillus oryzae*, autophagy mediates the degradation of basal cell components, such as entire nuclei, which appears to facilitate hyphal tip growth through the recycling of nutrients that are generated by nuclear degradation (Shoji et al., 2010). As the fungal cells are multinucleate, the loss of some nuclei can occur without detrimental effects. This process has been defined as macroautophagy on the basis of experiments that used EGFP-tagged Atg8 to follow autophagosomal structures (Shoji et al., 2010). These authors show that crescent-like autophagosomal precursors that are in close proximity to the targeted nuclei mature into large ring-like autophagosomes (1–2 µm in diameter) that then sequester whole nuclei. Subsequently, nuclear material is dispersed throughout neighboring vacuoles, suggesting that autophagosomes fuse with vacuoles for macroautophagy-mediated degradation of whole nuclei (see Poster). Because virtually all of the large autophagosomes contain at least one nucleus, they are likely to be specifically dedicated to degradation of nuclei (Shoji et al., 2010).

Macroautophagy-mediated degradation of the nucleus also occurs in the rice blast fungus, *Magnaporthe oryzae*. To be able to infect rice plants, *M. oryzae* forms a specialized structure, the appressorium, which generates immense intracellular turgor pressure that results in the direct rupture of the plant cuticle and, thereby, facilitates fungal entry and colonization of plant tissue (Liu et al., 2012). Appressorium development involves a number of steps, one of which is nuclear degeneration in the spore from which the appressorium develops (see Poster) (Veneault-Fourrey et al., 2006; He et al., 2012). Although, *M. oryzae* possesses homologs of Vac8 and Tsc13, components of a putative PMN pathway, an Nvj1 homolog has not been identified and there is no evidence for the formation of NV junctions and for PMN activity. Moreover, both *M. oryzae* Vac8 and Tsc13 are dispensable for nuclear breakdown during plant infection (He et al., 2012). In contrast to PMN, nuclear degradation in *M. oryzae* is dependent on core autophagy genes, such as *M. oryzae ATG1* or *ATG4* (Kershaw and Talbot, 2009). In contrast to large ring-like autophagosomes that mediate the degradation of *A. oryzae* nuclei, only smaller, more punctate autophagosomes surround the target nucleus during appressorium formation in *M. oryzae* (He et al., 2012). Moreover, the nucleus does not appear to be fully degraded, suggesting that nuclear breakdown might be performed by distinct macroautophagy-dependent processes in filamentous fungi (He et al., 2012).

Programmed nuclear death of the old macronucleus in *T. thermophila*

T. thermophila contains two functionally distinct nuclei in one cell. The polyploid macronucleus divides by amitosis (i.e. through separation); it is a site of mRNA synthesis and governs the somatic phenotype of the cell. The diploid micronucleus is transcriptionally inactive and serves as the germ nucleus. During sexual reproduction, a complex process of nuclear differentiation and degradation takes place. The degradation of the 'old' macronucleus by programmed nuclear death (PNuD) shows some characteristics of mammalian apoptosis (Davis et al., 1992; Lu and Wolfe, 2001; Liu and Yao, 2012) but autophagy is also crucial (Lu and Wolfe, 2001; Akematsu et al., 2010; Liu and Yao, 2012). Two different models for the role of autophagy have been proposed (models 1 and 2, see Poster). In one model, autophagosomes do not appear to form (Akematsu et al., 2010); during degradation of the old macronucleus, the nuclear envelope undergoes significant changes and exhibits characteristics of autophagosome membranes and also exposes certain sugars and phosphatidylserine on the outer membrane surface (Akematsu et al., 2010). The authors interpreted these changes as destruction signals that facilitate the subsequent fusion of the target nucleus with lysosomes (see Poster). By contrast, the second model suggests a coordinated formation of autophagosomes that surround the target nucleus (Lu and Wolfe, 2001; Liu and Yao, 2012). In the initial study (Lu and Wolfe, 2001), the vesicles were not well characterized, but in the later study (Liu and Yao, 2012), it was observed that two orthologs of Atg8 were involved, supporting the involvement of autophagosomes (see Poster). Additional support for the role of these Atg8 proteins as bona fide components of autophagosomes comes from *atg8*-knockout mutant cells, which show a pronounced delay in nuclear degradation (Liu and Yao, 2012). However, further studies are required to fully understand the precise autophagic mechanism involved.

Nucleophagy in mammalian cells

A role for autophagy in the degradation of the entire nucleus or nuclear components (see Poster), such as chromatin in mammalian cells has been suggested for many years. However, only little or no details were available with regard to the particular mode of nucleophagy involved (Mijaljica et al., 2007; Mijaljica et al., 2010).

The first definitive report of nucleophagy in mammalian cells was based on electron microscopy (EM)

images that showed the presence of perinuclear vesicular structures in cells from nuclear envelopathies and emerinopathies that are caused by mutations in the genes encoding A-type lamins (*LMNA*) and emerin, respectively (Park et al., 2009). Consequently, mouse embryonic fibroblasts (MEFs) bearing an *Lmna* mutation and expressing GFP-labeled LC3 exhibit round, LC3-positive structures in very close proximity to the nucleus. These structures were identified as autophagosomes based on their incorporation of GFP-labeled LC3, the mammalian homolog of Atg8 (a marker of autophagosomes), and their colocalization with the core autophagy proteins ATG5, ATG16L and ATG9 (Park et al., 2009). In addition, the lysosomal marker protein LAMP2 was also found to colocalize to some of these structures, suggesting the fusion of autophagosomes with lysosomes to form autophagolysosomes. The colocalization of GFP–LC3 with histones H1 and γH2AX suggests the presence of chromatin within some autophagosomes. Importantly, a decreased intensity of staining by DAPI is consistent with chromatin degradation in these compartments.

Micronuclei are mainly displaced chromosomes or chromosome fragments that are enclosed by nuclear membrane (Fenech et al., 2011), and their formation can be enhanced following exposure to genotoxic compounds (Erenpreisa et al., 2012). Recently, it has been shown that autophagy is also involved in the elimination of micronuclei (Rello-Varona et al., 2012). Here, micronuclei were found to contain chromatin, and a few of these (2–5%) colocalize (adjacent to or enveloped by) to LC3-positive vesicles. Colocalization with LC3 is lost after knockdown of the core autophagy genes *ATG5* or *ATG7*, confirming that the chromatin and LC3-positive structures are indeed autophagosomes. The autophagic LC3-positive micronuclei also contain the autophagy adaptor p62 (SQSTM1), which does not localize to non-autophagic LC3-negative micronuclei. In addition, LC3-positive micronuclei exhibit signs of nuclear envelope degradation, contain foci of γH2AX-positive DNA, a marker of DNA damage, and stain less intensively for chromatin markers. By contrast, LC3-negative micronuclei are surrounded by an intact membrane and only rarely show signs of DNA damage (Rello-Varona et al., 2012). Presumably, autophagic degradation of micronuclei might contribute to genome stabilization and protect against chromosomal instability (Boya and Codogno, 2012).

A subsequent study addressed the low occurrence of LC3-positive micronuclei and suggested that it derives from the DNA repair processes that might occur within micronuclei (Erenpreisa et al., 2012). The authors suggest that LC3-positive vesicles represent a failure of DNA repair attempts in micronuclei, so they are subsequently removed by autophagy (Erenpreisa et al., 2012). This is consistent with the reduced presence of the DNA damage marker γH2AX and chromatin staining with Hoechst 33342 in these vesicles. They also suggest that some micronuclei maintain a connection with the nucleus by means of an extension of the nuclear envelope membranes that include one or more layers of heterochromatin of a diameter of 30 nm (so-called envelope-limited chromatin sheets, ELCS). This connection allows the micronuclei to bridge to new nuclear envelope sites where their DNA content can be repaired and reincorporated into the nucleus, and might have been the LC3-negative micronuclei (Rello-Varona et al., 2012). This model further suggests that, if repair and reintegration fails, these micronuclei would 'bud' from the nucleus (i.e. lose their connection) and be degraded by autophagy (Erenpreisa et al., 2012). Further studies of the physical connections between micronuclei and the nucleus are clearly needed to understand any ongoing repair events.

The links of nucleophagy to human disease are only just emerging (Box 1). Although the formation of micronuclei has been observed in many disease situations, such as Bloom syndrome (Yankiwski et al., 2000) and cancer (Luzhna et al., 2013), evidence has only emerged recently that micronuclei can be degraded by autophagy (Rello-Varona et al., 2012; Erenpreisa et al., 2012) and the full significance of this process in the context of disease pathology and progression remains to be investigated.

Future perspectives

As discussed here, nucleus-derived cargo can be subjected to degradation by autophagic processes we collectively refer to as nucleophagy. However, our knowledge of nucleophagy is incomplete and a number of unanswered questions remain (Box 2), particularly with regard to the divergent modes of nucleophagy in lower eukaryotes.

It is unlikely that a specialized process of nucleophagy, such as PMN is required

Box 1. Emerging links between nucleophagy and human disease

Very recent observations suggest that senescent cells can process chromatin by extrusion of fragments of chromatin from the nucleus into the cytoplasm. These fragments are enriched for γH2AX (a DNA damage marker) and presumably represent a mechanism for moving damaged genome fragments out of the nucleus and targeting them to the autophagy machinery for degradation, thereby contributing to stability of senescence and tumor suppression. Evidence for a role for autophagy comes from the colocalization of chromatin fragments with the autophagy adaptor p62/SQSTM1 and lysosomes (Ivanov et al., 2013).

Some 25% of cases of Diamond Blackfan Anemia (DBA), a severe hypoplastic anemia, are linked to heterozygous mutations in the gene encoding ribosomal protein S19 and a haploinsufficiency for this protein. Depletion of RPS19 results in the accumulation of aberrant pre-40S particles, which are retained in the nucleus. Expression of DBA patient-associated mutations in yeast Rps19p, leads to defects in the processing of pre-RNA similar to those observed in mammalian cells (Léger-Silvestre et al., 2005). EM of yeast cells depleted of Rps19 using a repressible promoter shows what appears to be the vacuole engulfing the nucleolus (Léger-Silvestre et al., 2005), suggesting a PMN-like process, but this remains to be definitively established. If confirmed, it would indicate a role for autophagy in the pathogenesis of disease (Mijaljica et al., 2010).

Nucleophagy has also been observed in a small percentage of wild-type cells (Park et al., 2009), which suggests that the autophagic degradation of nuclear components is part of the normal regulation of cellular homeostasis that is achieved by autophagy. A beneficial role for nucleophagy is emphasized by the observation that inhibition of autophagy in MEFs carrying *Lmna* mutations leads to the accumulation of nuclear abnormalities, such as an irregularly shaped nucleus or nuclear envelope disruption, and reduced cell viability (Park et al., 2009).

Journal of Cell Science

Box 2. Unanswered questions on nucleophagy

Lower eukaryotes

- PMN: why does fusion of the vacuolar membrane component of the PMN vesicle depend on Atg genes?
- Late nucleophagy: what is the identity of, and manner by which, nuclear material enters the yeast vacuole?
- Although both PMN and late nucleophagy can be induced by starvation, a full understanding of the physiological relevance of both processes remains to be established.
- PND: how is the vacuolar lytic activity selectively targeted to only those nuclei not encapsulated into spores? Is it dictated by the nature of the different environments in which the nuclei are located, particularly in relation to being encapsulated or not encapsulated by a spore membrane?
- For PNuD in filamentous fungi, a genetic dissection of the mechanism in *A. oryzae* would shed light on the following questions. Which components of the classical macroautophagy machinery might be required for the formation of the atypical ring-like autophagosome structures? Is it a macroautophagy process distinct from the formation of the more typical punctate autophagosomes observed in *M. oryzae*? Can both processes occur in the same cell either coincidentally or sequentially?

Mammalian cells

The formation of micronuclei might be a pathway for 'making available' nuclear material for degradation by autophagy. The principal question the field needs to address is how individual micronuclei, particularly those in which failed DNA repair has occurred are selected for degradation by autophagy or nucleophagy (Boya and Codogno, 2012).

in mammalian cells, because, unlike yeast cells, which undergo closed mitosis, either macroautophagy or microautophagy could be utilized during S phase when the nuclear membrane has been disassembled and nuclear material is accessible (Mijaljica et al., 2010). A specialized mode of nucleophagy has been suggested to be more important for long-lived amitotic cells, such as neurons (Kvam and Goldfarb, 2007). In these cells, the potential nuclear material that is destined for destruction is sequestered for macro- or micro-autophagy by the intact nuclear membrane, which is not dismantled for the purpose of chromosome replication and cell division. In this context, the formation of micronuclei might be a means to make nuclear material available for degradation by autophagy. To date, however, there are only very few reports for the presence of micronuclei in amitotic cells that have not been subjected to radiation or genotoxins, and they offer no clues with regard to the likely importance of micronuclei formation and degradation in physiological settings.

Despite the number of unanswered questions, the future holds the promise of significant progress in our understanding of the mechanisms and physiological significance of nucleophagy. Addressing and answering these questions in mammalian cells will contribute to a better understanding of how dysregulation of nucleophagy contributes to disease.

Funding

The work of our laboratory was supported by the Australian Research Council [grant number DP0986937 to R.J.D.].

A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.133090/-/DC1.

References

Akematsu, T., Pearlman, R. E. and Endoh, H. (2010). Gigantic macroautophagy in programmed nuclear death of Tetrahymena thermophila. *Autophagy* **6**, 901-911.

Boya, P. and Codogno, P. (2012). Micronucleophagy: a new mechanism to protect against chromosomal instability? *Cell Cycle* **11**, 645-646.

Davis, M. C., Ward, J. G., Herrick, G. and Allis, C. D. (1992). Programmed nuclear death: apoptotic-like degradation of specific nuclei in conjugating Tetrahymena. *Dev. Biol.* **154**, 419-432.

Dawaliby, R. and Mayer, A. (2010). Microautophagy of the nucleus coincides with a vacuolar diffusion barrier at nuclear-vacuolar junctions. *Mol. Biol. Cell* **21**, 4173-4183.

Eastwood, M. D., Cheung, S. W., Lee, K. Y., Moffat, J. and Meneghini, M. D. (2012). Developmentally programmed nuclear destruction during yeast gametogenesis. *Dev. Cell* **23**, 35-44.

Eastwood, M. D., Cheung, S. W. and Meneghini, M. D. (2013). Programmed nuclear destruction in yeast: self-eating by vacuolar lysis. *Autophagy* **9**, 263-265.

Erenpreisa, J., Huna, A., Salmina, K., Jackson, T. R. and Cragg, M. S. (2012). Macroautophagy-aided elimination of chromatin: sorting of waste, sorting of fate? *Autophagy* **8**, 1877-1881.

Fenech, M., Kirsch-Volders, M., Natarajan, A. T., Surralles, J., Crott, J. W., Parry, J., Norppa, H., Eastmond, D. A., Tucker, J. D. and Thomas, P. (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis* **26**, 125-132.

Geng, J., Klionsky, D. J. and Guan, J.-L. (2013). Autophagy in fungi and mammals. In: *The Encyclopedia Of Biological Chemistry*, Vol. 1 (ed. W. J. Lennarz and M. D. Lane), pp. 149-154. Waltham, MA: Academic Press.

He, M., Kershaw, M. J., Soanes, D. M., Xia, Y. and Talbot, N. J. (2012). Infection-associated nuclear degeneration in the rice blast fungus Magnaporthe oryzae requires non-selective macro-autophagy. *PLoS ONE* **7**, e33270.

Ivanov, A., Pawlikowski, J., Manoharan, I., van Tuyn, J., Nelson, D. M., Rai, T. S., Shah, P. P., Hewitt, G., Korolchuk, V. I., Passos, J. F. et al. (2013). Lysosome-mediated processing of chromatin in senescence. *J. Cell Biol.* **202**, 129-143.

Kershaw, M. J. and Talbot, N. J. (2009). Genome-wide functional analysis reveals that infection-associated fungal autophagy is necessary for rice blast disease. *Proc. Natl. Acad. Sci. USA* **106**, 15967-15972.

Krick, R., Muehe, Y., Prick, T., Bremer, S., Schlotterhose, P., Eskelinen, E. L., Millen, J., Goldfarb, D. S. and Thumm, M. (2008). Piecemeal microautophagy of the nucleus requires the core macroautophagy genes. *Mol. Biol. Cell* **19**, 4492-4505.

Krick, R., Mühe, Y., Prick, T., Bredschneider, M., Bremer, S., Wenzel, D., Eskelinen, E. L. and Thumm, M. (2009). Piecemeal microautophagy of the nucleus: genetic and morphological traits. *Autophagy* **5**, 270-272.

Kvam, E. and Goldfarb, D. S. (2006). Nucleus-vacuole junctions in yeast: anatomy of a membrane contact site. *Biochem. Soc. Trans.* **34**, 340-342.

Kvam, E. and Goldfarb, D. S. (2007). Nucleus-vacuole junctions and piecemeal microautophagy of the nucleus in *S. cerevisiae*. *Autophagy* **3**, 85-92.

Léger-Silvestre, I., Caffrey, J. M., Dawaliby, R., Alvarez-Arias, D. A., Gas, N., Bertolone, S. J., Gleizes, P. E. and Ellis, S. R. (2005). Specific role for yeast homologs of the diamond blackfan anemia-associated rps19 protein in ribosome synthesis. *J. Biol. Chem.* **280**, 38177-38185.

Liu, M. L. and Yao, M. C. (2012). Role of ATG8 and autophagy in programmed nuclear degradation in Tetrahymena thermophila. *Eukaryot. Cell* **11**, 494-506.

Liu, X.-H., Gao, H.-M., Xu, F., Lu, J.-P., Devenish, R. J. and Lin, F.-C. (2012). Autophagy vitalizes the pathogenicity of pathogenic fungi. *Autophagy* **8**, 1415-1425.

Lu, E. and Wolfe, J. (2001). Lysosomal enzymes in the macronucleus of Tetrahymena during its apoptosis-like degradation. *Cell Death Differ.* **8**, 289-297.

Luzhna, L., Kathiria, P. and Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. *Front. Genet.* **4**, 131.

Mijaljica, D., Prescott, M. and Devenish, R. J. (2007). Nibbling within the nucleus: turnover of nuclear contents. *Cell. Mol. Life Sci.* **64**, 581-588.

Mijaljica, D., Prescott, M. and Devenish, R. J. (2010). The intricacy of nuclear membrane dynamics during nucleophagy. *Nucleus* **1**, 213-223.

Mijaljica, D., Prescott, M. and Devenish, R. J. (2012). A late form of nucleophagy in Saccharomyces cerevisiae. *PLoS ONE* **7**, e40013.

Millen, J. I., Krick, R., Prick, T., Thumm, M. and Goldfarb, D. S. (2009). Measuring piecemeal microautophagy of the nucleus in Saccharomyces cerevisiae. *Autophagy* **5**, 75-81.

Park, Y. E., Hayashi, Y. K., Bonne, G., Arimura, T., Noguchi, S., Nonaka, I. and Nishino, I. (2009). Autophagic degradation of nuclear components in mammalian cells. *Autophagy* **5**, 795-804.

Rello-Varona, S., Lissa, D., Shen, S., Niso-Santano, M., Senovilla, L., Mariño, G., Vitale, I., Jemaá, M.,

Harper, F., Pierron, G. et al. (2012). Autophagic removal of micronuclei. *Cell Cycle* **11**, 170-176.
Roberts, P., Moshitch-Moshkovitz, S., Kvam, E., O'Toole, E., Winey, M. and Goldfarb, D. S. (2003). Piecemeal microautophagy of nucleus in Saccharomyces cerevisiae. *Mol. Biol. Cell* **14**, 129-141.
Shoji, J. Y., Kikuma, T., Arioka, M. and Kitamoto, K. (2010). Macroautophagy-mediated degradation of whole nuclei in the filamentous fungus Aspergillus oryzae. *PLoS ONE* **5**, e15650.
van Doorn, W. G. and Woltering, E. J. (2005). Many ways to exit? Cell death categories in plants. *Trends Plant Sci.* **10**, 117-122.
van Doorn, W. G. and Woltering, E. J. (2010). What about the role of autophagy in PCD? *Trends Plant Sci.* **15**, 361-362.
Veneault-Fourrey, C., Barooah, M., Egan, M., Wakley, G. and Talbot, N. J. (2006). Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science* **312**, 580-583.
Yankiwski, V., Marciniak, R. A., Guarente, L. and Neff, N. F. (2000). Nuclear structure in normal and Bloom syndrome cells. *Proc. Natl. Acad. Sci. USA* **97**, 5214-5219.