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Review

Nibbling within the nucleus: turnover of nuclear contents

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Abstract. As the site of gene expression and regulation, the nucleus is the control center of the cell. It might be thought that degradation of nuclear contents is strictly 'off-limits,' given the importance of the genetic information contained within the nucleus, but it has recently been reported that partial degradation of the nucleus may occur in yeast. Here we summarize the evidence for the degradation and quality control of proteins found with the nucleus and its compartments, and of nucleic acids that may occur under certain specific conditions. Only under certain special conditions such as differentiation of the lens are the entire nuclear contents degraded.

Keywords. Autophagy, degradation, exosome, nucleoporin, nuclear compartment, nucleus, piecemeal microautophagy, protein quality control.

The nucleus – control center of the cell

The nucleus is usually the most prominent structure within a eukaryotic cell, being the largest of the membrane-bound organelles. It has two main functions. First, the nucleus is the repository for the cell's genetic information, in the form of the chromosomes. Each time the cell divides, the genetic information is copied such that each new cell receives a copy. Second, the nucleus is the site of gene expression and gene regulation. The cell nucleus ensures that the variety of complex and specialized molecules the cell requires for its varied functions are supplied in the amounts needed [1]. In other words, the cell nucleus is the center for direction and coordination of the cell's reproductive and metabolic activities.

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Nuclear compartments and their dynamics

The cell nucleus can be considered to contain several distinct compartments [1–4] (Table 1). These include the nucleolus [5–10], the splicing-factor compartments (SFCs) [4, 10–13], the Cajal bodies [4, 8, 14–16], the promyelocytic leukemia oncoprotein (PML) bodies [17–22], nuclear envelope [23-29] and a rapidly growing family of small dot-like nuclear bodies [5, 9]. All these compartments are found in the nucleoplasm, which itself can be considered as a compartment [1]. These compartments are characterized by the absence of delineating membranes, a feature that distinguishes them clearly from their cytoplasmic organellar counterparts. Nevertheless, their assignment as compartments is made for the following reasons. First, they contain defining subsets of resident proteins. Although proteins are highly mobile within the nucleus [1], nuclear compartments appear to contain a stable composition of protein types, even though the individual protein components of the compartment may

Table 1. Nuclear compartments and their degradation.

Compartment	Contents and structure	Known or putative function (s)	References	Possible degradative mechanisms; Refs.
Nucleolus	fibrillar centers, dense fibrillar components, granular components, chromatin inclusions	Ribosome biogenesis and assembly, maturation of nonribosomal RNAs, nuclear export, sequestering regulatory molecules, modifying small RNAs, controlling aging	1, 5–8	Apoptosis, Autophagy [23–26, 75]
Splicing factor compartments	interchromatin granule clusters, perichromatin fibrils, pre-mRNA splicing factors, ribosomal proteins and transcription factors (~200 proteins), small nuclear RNAs	Transcription and splicing, recycling and reactivation of splicing factors, storage and assembly of spliceosomal components	4, 8–13	proteasomes [34—37]
Cajal body	nuclear protein p80-coilin, factors involved in pre-mRNA splicing, pre-rRNA processing, and histone pre-mRNA processing, all three RNA polymerases	assembly and modification of RNA-processing complexes, regulation of transcription at specific loci, sites for assembly of the transcription and processing machinery	4, 8, 14–16	Exosome [43—45]
Promyelocytic leukemia oncoprotein (PML)	PML protein, Bloom protein, topoisomerase TopoIII, cyclin-dependent protein kinase p27Kip1, p53, beta-catenin	transcription, DNA repair, viral defence, stress, cell cycle regulation, proteolysis and apoptosis	17–22	Proteasomes [43]
Nuclear envelope	inner and outer nuclear membrane, nuclear pore complexes, nucleoporins (e.g. Nup153, POM121, Nup159)	separates the nucleus from the cytoplasm, nuclear import and export	24–29	apoptosis, proteasomes, autophagy-independent process [24—29]
Small dot-like Bodies	diverse assortment of cellular components	sites of storage or sequestration of various nuclear components	1, 4	unknown

be continuously and rapidly exchanged. Secondly, they can be morphologically identified by light and electron microscopy, and all those listed in Table 1 except nuclear bodies have recently been visualized in living cells using fluorescent protein technology [1]. Finally, nucleoli and Cajal bodies can be biochemically isolated in an enriched form [1, 30–33].

Quality control in the nucleus – destruction for the sake of overall function

Aberrant proteins arise as a consequence of mutations, transcriptional and translational errors, incorrect folding, imbalanced subunit synthesis, improper trafficking or damage caused by environmental conditions or metabolic byproducts [33–34]. To minimize harmful effects from aberrant proteins, the cell possesses a set of protein quality control (PQC) systems that operate in distinct ways. PQC degradation systems have been identified in the cytoplasm, the secretory pathway and mitochondria. In both the cytoplasm and the endoplasmic reticulum (ER), PQC degradation is primarily brought about by proteasomal degradation of proteins marked by protein-ubiquitination complexes [34–37]. PQC degradation of cytoplasmic and ER proteins can also occur via transport to the lysosomes/vacuole [38]. In the mitochondria, proteases localized in the intermembrane space or in the matrix function in PQC degradation [39].

In contrast, less is known about PQC in the nucleus. Protein synthesis does not occur in the nucleus, so that the cell does not have to contend with protein production defects in the nucleus except those represented by imported defective proteins that have eluded cytoplasmic PQC systems. In addition, nuclear proteins can be damaged by similar stresses that damage proteins in other cellular compartments; thus, the cell must also contend with aberrant proteins that arise by such means. An accumulation of aberrant proteins in the nucleus can have deleterious effects. For example, such an accumulation likely underlines the pathology of oculopharyngeal muscular dystrophy (OPMD) and Huntington's disease [40–41]. At least some regulated degradation apparently occurs in the nucleus via ubiquitination and nuclear-localized proteasomes [42], implying that the nucleus has the potential machinery for PQC degradation. A recent report suggests that the ubiquitin-proteasome system

is not only abundant in the cell nucleus, but may play a major role in regulation of nuclear structure and function by protein degradation in distinct subnuclear compartments [43]. Cell fractionation and microinjection of fluorogenic substrates was used to detect significant proteasomal activity in nucleoplasmic cell fractions, whereas activity was not detectable in nucleoli or in nuclear envelope protein fractions of human cells. An advantage of proteolysis within the nucleus would be to enhance the regulation of processes involved in gene expression, since proteins engaged in such functions could be degraded *in situ* rather than first having to be exported to the cytoplasm.

In yeast (*Saccharomyces cerevisiae*), the San1p protein, a ubiquitin-protein ligase, is nuclear localized and requires nuclear localization to function. Recently, it was reported that in conjunction with the ubiquitin-conjugating enzymes, Cdc34p and Ubc1p, San1p targets four distinct mutant nuclear proteins (Cdc68-1p, Sir4-9p, Cdc13-1p and Sir3-8p; proteins involved in gene transcription and telomere capping) for ubiquitination and proteasome-predicted destruction. Loss of the SAN1 gene results in a chronic stress response in yeast cells, underscoring its role in PQC [34].

Glickman and Ciechanover [44] reported that in mammalian cells, some proteins such as p53, β-catenin and p27kip1 are degraded mainly in the nucleus by the nuclear proteasomal degradation pathway. However, β-catenin can also be directed to cytoplasmic ubiquitin-proteasome degradation. From these cases, it is clear that both routing of the substrate and compartment-specific activity of components of the ubiquitin system play important roles in governing the stability of different proteins. The physiological significance, however, of regulation via nuclear-cytoplasmic shuttling in this context remains to be elucidated.

Like the proteasome, the exosome is a multi-protein complex present in both the nucleus and cytoplasm. The exosome performs both processing of some RNA substrates to shorter forms (e.g. trimming reactions that produce the mature 3′ end of several stable RNAs) and the complete degradation of other substrates (e.g. the 5′ external transcribed spacer region of the primary transcript for rRNA) [45]. Mutations in some exosome components have been observed to lead to the accumulation of polyadenylated transcripts in the nucleus as assessed by *in situ* hybridization [46]. The exosome has also been suggested to function in the degradation of pre-ribosomal RNA (rRNA) transcripts that cannot be properly processed [47]. Important issues for future work will be to understand the structure and organization of the exosome, how different substrates are recognized and sequestered, and which of the protein components of the complex are involved in the degradation process in the nucleus.

In addition to aberrant mRNAs, normal mRNAs are also reported to undergo decay in the nucleus by a degradation system designated DRN (degradation of mRNA in the nucleus). Sherman and colleagues [48] propose that all mRNAs in *S. cerevisiae* associated with the nuclear cap-binding complex, which includes Cbc1p (CBP80) and Cbc2p (CBC20), have two possible fates. First, they may be subjected to export (i.e. they escape degradation) or, alternatively, they may be retained within the nucleus. If retained, they are directed to a site for degradation by Rrp6p (a 3′-to-5′ exonuclease) in combination with Rat1p (a 5′-to-3′ exonuclease). Particular mRNAs, e.g. SKS1 mRNA, are highly susceptible to DRN in normal yeast strains owing to preferential nuclear retention.

Degradation of DNA and nuclear proteins during apoptosis, development and other cellular processes

Recent studies of apoptotic DNA degradation *in vitro* and *in vivo* indicate that two independent systems are involved in DNA degradation during programmed cell death [49–56]. The first system is a cell-autonomous one that functions in dying cells, while the other system takes place in macrophages after dying cells have been engulfed by phagocytes. When cells receive apoptotic stimuli, a cascade of caspases (central executioners of apoptosis) is activated, which leads to death following cleavage of various cellular proteins, and the degradation of DNA by CAD/DFF (caspase-activated DNase or DNA fragmentation factor). Dying cells expose phosphatidylserine (PS) as an "eat me" signal on their surface that is recognized by macrophages. Following the engulfment of dead cells by phagocytosis and the subsequent fusion of phagocytic vesicles with lysosomes, the DNA of the dead cells is degraded by DNase II [56].

Nuclear apoptosis is manifested as chromatin condensation, nucleosomal fragmentation, perturbation of the nuclear envelope and clustering of nuclear pores [23–26]. Proteins of the nuclear pores (NUP153), the inner nuclear membrane (LBR and Lap2) and the nuclear lamina (lamin B) are targets of specific executioner caspases [28, 29]. Hallberg and colleagues [23] have reported the sequential degradation of proteins from the nuclear envelope during apoptosis in buffalo rat liver cells. They found that the nuclear pore membrane protein POM121 was eliminated significantly more rapidly than NUP153 and lamin B. Also, they showed that disappearance of NUP153 and lamin B was coincident with onset of DNA fragmentation and clustering of nuclear pores.

By contrast, the peripheral nuclear pore complex (NPC) protein p62 was degraded much later. The results suggest that degradation of POM121 may be an important early step in propagation of nuclear apoptosis and that elimination of proteins from the nuclear envelope may be preferential [23].

It has been recently reported that the permeability of the yeast nuclear envelope (NE) increases during hydrogen peroxide-induced cell death. This process is analogous to increases in nuclear pore complex permeability observed during apoptosis in mammalian tissue culture cells [57–59]. Goldfarb and colleagues [60] reported that a number of yeast nucleoporins (molecular components of the NPC) are degraded during hydrogen peroxide-induced cell death, but that this occurs late, after significant increases in NE permeability. The peripheral nucleoporins (Nup1p and Nup159p) were degraded earlier than the core nucleoporins (Nsp1p, Nup100p and Nup116p). It was suggested that the degradation of different nucleoporins is dependent on the vacuolar endopeptidase, Pep4p, the yeast cathepsin D homologue. It has been well documented in certain mammalian cell death models that lysosomal cathepsin D mediates apoptosis induced by oxidative stress by various compounds such as hydrogen peroxide [61–64]. Although intriguing, it is possible that the degradation of nucleoporins by cathepsin D homologues during cell death is an adaptive process, but this requires further investigation.

The observations discussed above clearly indicate that cleavage by caspases of some proteins of the NE and the NPC contributes to apoptosis in the nucleus. The evidence supporting a key role for nucleo-cytoplasmic transport (both import and export) in the degradation of such nuclear components by regulating the accessibility of key players in apoptosis to the nucleus has recently been reviewed by Fahrenkrog [65].

Nuclear degradation occurs in the developmental processes of erythropoiesis and lens cell differentiation (Fig. 1). The nuclei of erythrocytes and lens fiber cells are removed during terminal differentiation into mature cells. In erythropoiesis, nuclei are expelled from the erythroid precursor cells [56]. As this process does not involve nuclear degradation as a primary event it is not considered further here. The importance of the associated degradation of DNA by DNaseII is emphasized by the observation that in the mouse DNase II-null-mutant, embryos suffer from severe anemia due to defective erythropoiesis [56, 66–67]. Elimination of the nucleus (and other cellular organelles) from the lens occurs during both late embryogenesis and neonatal development. In late embryogenesis organelles are eliminated from fiber cells in the central regions of the lens. During neonatal development, epithelial cells located at the apical equator of the lens migrate towards the center of the lens and concomitantly start to differentiate into fiber cells, losing their organelles in the process [56].

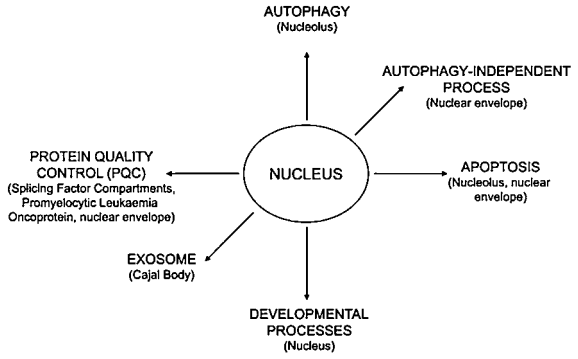

Figure 1. Possible routes and developmental processes involved in the degradation of the nucleus and its compartments. On the one hand, the nucleus contains its own quality control machinery (proteasomes [24–29, 34–37, 43] and the exosome protein complex [43-45]). On the other, the nucleus can be at least partially degraded through autophagy [75], apoptosis [23–29] and an autophagy-independent degradation pathway [24–29]. In addition, the nucleus and its compartments are modified or lost during developmental processes [56, 67] such as lens differentiation.

In the eye, the degradation of DNA is not carried out by CAD, since this enzyme is apparently not expressed. Furthermore, there is no abnormality in the eye of CAD-null mice [68]. On the other hand, DNase II-like acid DNase (DLAD) is expressed specifically in human and murine lens cells. Mice deficient in the DLAD gene were incapable of degrading DNA during lens cell differentiation, and undigested DNA accumulated in the cytoplasm of the fiber cells. DLAD-null mice developed weak cataracts and their response to light was severely reduced, confirming that nuclei must be lost from fiber cells to ensure the transparency of the lens. If other organelles are left undigested in the lens, they may also cause cataracts. Some human cataracts thus may be caused by a genetic defect that impairs removal of organelles during lens cell differentiation. Localization of DLAD to lysosomes suggested that nuclei in the fiber cells are degraded during lens cell differentiation via the process of autophagy [68]. This process might also account for the degradation/removal of other cellular organelles in the lens cell.

Mizushima and colleagues [67] have demonstrated that autophagy is not essential for either lens organelle-free zone (OFZ) formation, or for organelle elimination in erythroid cells, at least in newborn mice. Although their study suggests that autophagy is induced in the lens in normal mice, it might not be sufficient for the rapid elimination of entire organelles. In *Atg5*$^{-/-}$ mice (the ATG5 gene is generally

considered essential for autophagy), organelle degradation in the lens occurred normally [69]. Thus, it could be concluded that an autophagy-independent mechanism plays a primary role in OFZ formation. It could not be determined if autophagy was required for later differentiation steps because of the early lethality of *Atg5*$^{-/-}$ mice (within 10 h of birth). However, it is important to notice that this study excluded macroautophagy, but not other forms of autophagy, such as microautophagy. In addition, it was suggested that 15-lipoxygenase (15-LOX), a lipid-metabolizing enzyme, participates in organelle degradation in both lens cells and erythrocytes by permeabilization of organelle membranes, releasing luminal proteins and allowing the access of proteases to both luminal and integral membrane proteins. This enzyme is highly expressed in lens epithelial cells and outer fiber cells. Even if 15-LOX could trigger the elimination process, the organelle debris must be completely degraded afterwards. Since both lens epithelial cells and fiber cells have a fully functional ubiquitin-proteasome system, then it might be involved in the degradation of proteins derived from organelles [70]. Furthermore, there is a possibility that an as yet unknown degradation system may participate in this process. Since organelle degradation is the fundamental process for these two types of cells, further analyses would be required to reveal its mechanism [71–72].

Autophagy-dependent degradation of the nucleus and its compartments

Organelle degradation by autophagy takes place through either a macroautophagic or a microautophagic route. During macroautophagy, double-membrane vesicles called autophagosomes enclose organelles, and these vesicles are then trafficked to the lysosomes (mammalian cells)/vacuole (yeast cells) for degradation. In contrast, microautophagy involves the direct sequestration of cargo via an invagination of the vacuolar membrane, and therefore lacks vesicular intermediates [73]. The nucleus has been largely ignored as a possible target for autophagy, probably because of its importance as the cellular command center containing the genetic information that has to be protected from exposure to any hostile environment. In the case of autophagy, this would be the acidic hydrolases of the lysosomal/vacuolar compartment. If the destructive contents of the lysosomes/vacuole were released within the nucleus, they would create havoc with dire consequences for the cell.

In *Tetrahymena thermophila,* the elimination of the old (and nonfunctional) macronucleus as part of completion of the conjugative process apparently relies on a two-stage process of apoptotic induction followed by autophagic completion [74]. Apoptotic induction is evidenced by hallmarks of apoptosis (e.g. chromatin condensation, caspase activation and DNA cleavage into nucleosome-sized fragments). The second stage, autophagy, is evidenced by the apparent acidification of the condensed old macronucleus, the localization of acid phosphatase within this organelle as it is degraded and the electron microscopic demonstration of membranous vesicles enclosing the condensed nuclear structure.

Clearly, the sole functional nucleus present in most cells cannot be completely degraded. A possible role of autophagy in relation to functional nuclei would be the turnover of part of the nuclear contents to degrade aggregated or otherwise nonfunctional components. Indeed, such a process has recently been reported, whereby portions of the yeast nucleus are targeted for degradation by a novel form of selective microautophagy termed 'piecemeal microautophagy of the nucleus' (PMN) [75]. During PMN, small teardrop-like nuclear envelope blebs are engulfed by invaginations of the vacuolar membrane, pinched into the vacuole lumen and degraded by the luminal hydrolases.

PMN occurs through specific interactions between Vac8p on the vacuole membrane and Nvj1p in the NE. These nucleus-vacuole junctions expand and proliferate under conditions of nutrient depletion in yeast [76]. Our recent work (D. Mijaljica, M. Prescott, R.J. Devenish, unpublished data), has sought to follow nuclear turnover in yeast by using a fluorescent biosensor targeted to the nucleus. Thus, in wild-type yeast cells under starvation conditions (an induction signal for autophagy), we observe fluorescence in the vacuole, providing evidence for uptake of nuclear segments into the vacuole (Fig. 2).

An open question regarding PMN is how the selection of the material to be degraded, presumably only nonessential parts of the nucleus, is controlled. Goldfarb and colleagues [75] have provided evidence that portions of the NE and the granular nucleolus, containing such components as pre-ribosomes, can be taken up during PMN. They further reported that, in some instances, this nucleolar material appeared more concentrated than in the adjacent nucleolus, and noted that this could be related to the aggregation and remodeling known to occur within nucleoli under stress conditions. However, it is notable that not all the nuclear blebs contained nucleolar material, and it would be interesting to determine which, if any, of the other nuclear compartments might be degraded by PMN. How the regulated association between two dissimilar organellar membranes (nucleus and vacuole) is fine-tuned and regulated also remains to be established.

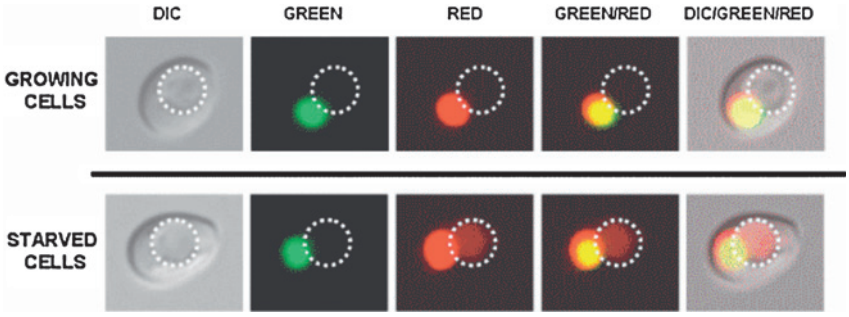

Figure 2. Vacuolar uptake of the nucleus during nitrogen starvation of yeast cells. Nomarski (DIC) and fluorescence (green, red and overlay) images of yeast (*S. cerevisiae*) cells under growing or nitrogen starvation conditions. The biosensor is fused to a nuclear localization signal (NAB35). The nucleus is delineated by both green (GFP) and red (RFP) fluorescence. The appearance of red fluorescence only in the vacuole is indicative of autophagic uptake of nuclear contents into the vacuole. The pH-insensitive RFP variant remains fluorescent within the acidic compartment of yeast cells (vacuole), whereas green fluorescence is not seen in the vacuole because the GFP component is pH sensitive at acidic pH. The location of the vacuole can be seen in the DIC images (and confirmed by independent labeling techniques), indicated by a white dotted circle.

Although the process of autophagy is universal among eukaryotic cells, PMN is at present unique to yeast, having not yet been identified in mammalian cells. A mechanistically similar process to PMN has been observed in Bloom's syndrome that results from mutations in a gene encoding a DNA helicase. This enzyme plays a role in uncoiling double-stranded DNA and appears to be essential for maintaining chromosome stability. The main feature of this process having similarity with PMN is the release of 'nuclear microvesicles' into the cytoplasm [77].

Concluding remarks

Prior to the description of PMN [75], there had been no indication that nuclear contents may be subject to specific degradation by autophagy. PMN appears to be an elegant solution enabling selected portions of the nuclear contents (but excluding the most vital material to the survival of the cell, the chromosomes) to be degraded. Even so, it remains to be seen whether PMN is a yeast curiosity. The significance of nuclear microvesicles in Bloom's syndrome remains unclear, but could provide an important starting point for building a new understanding concerning the generation and fate of nuclear-derived vesicles under a variety of cellular conditions.

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