CellPress

Review

Repair, Reuse, Recycle: The Expanding Role of Autophagy in Genome Maintenance

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(Macro)Autophagy is a catabolic pathway that delivers excess, aggregated, or damaged proteins and organelles to lysosomes for degradation. Autophagy is activated in response to numerous cellular stressors such as increased levels of reactive oxygen species (ROS) and low levels of cellular nutrients as well as DNA damage. Although autophagy occurs in the cytoplasm, its inhibition leads to accumulation of DNA damage and genomic instability. In the past few years, our understanding of the interplay between autophagy and genomic stability has greatly increased. In this review we summarize these recent advances in understanding the molecular mechanisms linking autophagy to DNA repair.

Introduction

Maintenance of genomic integrity is essential for organismal survival. DNA can be damaged by a plethora of extrinsic factors such as UV radiation, ionizing radiation, and chemical compounds as well as intrinsic factors such as free radicals generated as part of normal metabolism and mistakes in replication. This barrage of genotoxic insults results in the generation of an estimated 19 200 DNA lesions per day [1]. It is therefore necessary for cells to have effective mechanisms for sensing and repairing this damage to maintain their survival.

Like genomic integrity, maintenance of protein homeostasis is an essential requirement for cell and organismal survival. Thus, there are multiple pathways responsible for the turnover of damaged and unwanted proteins and organelles. The ubiquitin (Ub) proteasome system (UPS) is responsible for the degradation of short-lived, soluble proteins, is active in both the cytoplasm and the nucleus, and is thought to account for the majority of protein turnover in the cell. Tagging of target proteins with Ub chains makes the UPS highly selective. As a result of its ability to tightly control protein levels, the UPS has been shown to regulate numerous cellular processes such as the cell cycle, signal transduction, and DNA repair [2].

The term 'autophagy' translates from the Greek *auto* meaning 'oneself' and *phagy* meaning 'to eat' and describes evolutionarily conserved catabolic cellular degradation pathways involved in the delivery of cytoplasmic cargo to the lysosome. There are three main types of autophagy in eukaryotic cells: microautophagy (direct engulfment and degradation of portions of the cytoplasm via invagination of the lysosome [3]); chaperone-mediated autophagy (direct translocation of targeted proteins containing the KFERQ motif into the lysosome via the LAMP-2A receptor [4]); and macroautophagy (sequestration of cytoplasmic cargo in double-membrane vesicles called autophagosomes followed by transport along microtubules and fusion with the lysosome). Macroautophagy is the focus of this review and is herein referred to as autophagy. Similar to the UPS, cargo can be targeted for autophagy by the selective action of dedicated receptor proteins. Also as in the UPS, the main signal for autophagic degradation is the ubiquitination of cargo allowing its recruitment to the autophagosome by the receptors. Given the absence of

Trends

Recent studies have demonstrated the turnover of nuclear components such as nuclear lamina, chromatin, and DNA by autophagy and suggest that it plays an important role in maintaining genomic stability.

Loss/inhibition of autophagy gives rise to reduced DNA damage repair and increased cell death in response to genotoxic stress.

The accumulation of the autophagy receptor protein p62/SQSTM1 that results from inhibition/loss of autophagy leads to inhibition of double-strand break (DSB) repair through homologous recombination (HR).

Recently, progress has been made in unraveling the molecular mechanisms linking p62 and DSB repair. Nuclear p62 dampens HR through the inhibition of RNF168-mediated chromatin ubiquitination as well as targeting the HR proteins RAD51 and filamin A for degradation via the proteasome.

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autophagy in the nucleus and the previous belief that autophagy was a nonselective process, it was a long-held belief that the UPS was the sole degradation pathway impacting DNA damage repair. Recently, however, evidence has been accumulating to suggest that autophagy and DNA damage repair may be mechanistically linked despite occurring in spatially distinct cellular compartments. In this review we provide an overview of the current model of the core machinery of autophagy and the DNA damage response (DDR). We then focus on recent developments in our understanding of the role of autophagy in genome maintenance and DNA repair.

Autophagy

Autophagy plays a central role in the maintenance of cellular homeostasis and is active at basal, albeit varying, levels in all cells. Basal autophagic activity is dependent on cellular function as well as energy demand. Autophagy serves to dispose of damaged or unwanted proteins and organelles that accumulate as a consequence of normal cellular metabolism, like toxic protein aggregates and damaged mitochondria (reviewed in [5]). Its activity is governed by numerous sensors that detect changes to the intra- and extracellular environment, meaning that autophagy can be activated in response to various cell stresses such as starvation, hypoxia, mitochondrial damage, and pathogen infection. The primary purpose of this activation is to maintain cellular homeostasis and promote cell survival and disruption of the autophagic process has been implicated in the pathology of many human diseases, from cancer to age-related neurodegeneration [6].

Excessive activation of autophagy has also been observed in cells undergoing cell death. The exact role of this activation remains an area of debate as it is unclear whether cells die as a direct consequence of autophagic activation or whether autophagy is active in fatally stressed cells merely as a 'last-ditch', albeit failed, attempt to promote survival (reviewed in [7]). Recently, it has been suggested that autophagy may play a causative role in cell death in a pathway distinct from apoptosis or necroptosis [8]. Here, excessive activation of autophagy through treatment with autophagy-inducing peptides, starvation, or cerebral hypoxia–ischemia was shown to induce cell death via a mechanism dependent on cellular Na^+,K^+-ATPase. The prosurvival and possible pro-cell-death roles place autophagy at the center of the maintenance of cell and organismal hemostasis in response to various stresses.

During the process of autophagy, lipid membranes, the origin of which is debated, assemble in the cytosol giving rise to a nascent autophagic vesicle (the autophagosome) that engulfs cargo destined for degradation. Once the autophagosome is sealed, it is transported along microtubules and eventually delivers its cargo to the lysosome for degradation by forming a hybrid structure termed the autolysosome (Box 1). This process not only allows the removal of damaged, potentially toxic cellular components but also enables the release of basic molecules (e.g., amino acids, lipids) back to the cytoplasm for reuse in biosynthetic or catabolic processes as building blocks and a source of energy [9].

Initially believed to be a nonselective process, autophagy is now known to show specificity towards various intracellular substrates. This specificity is mediated by autophagic receptors such as the prototypical member of the family p62/SQSTM1 as well as several functionally related proteins [10]. Most of the receptor proteins contain Ub-binding domains, allowing them to interact with cargo tagged for autophagic degradation with Ub chains including Lys63–poly-Ub and other types of linkage [11]. While not considered to be required for autophagosomal biogenesis itself, receptor proteins mediate the docking of autophagic membranes to cargo and promote its sequestration by the nascent autophagosome. This function is mediated by LC3-II-interacting region (LIR) motifs [12], which allow interaction with autophagy (Atg)8-family proteins on the inner surface of the autophagosome (Box 1). Receptor-mediated selective autophagy can target various subcellular components and can be classified intosubtypes including aggrephagy

Box 1. Autophagic Machinery

The process of autophagosome formation is carefully orchestrated by over 30 core Atg proteins and a plethora of associated regulatory components. The initiation step in the formation of the nascent autophagic vesicle (also called a phagophore or an isolation membrane) requires the activity of the Atg1 (or ULK1) complex (Figure I). The protein kinase ULK1 is activated in response to nutrient deprivation, which is mediated by the suppression of an upstream protein kinase, mTOR complex 1 (mTORC1). Inhibition of mTORC1 relives ULK1 from its inhibitory phosphorylation allowing it to form a complex with the Atg13, FIP200, and Atg101 proteins [68]. The nucleation event, which follows initiation, is driven by a second key protein complex involving a phosphatidylinositol 3-kinase (PI3K), Vps34. Together with Vps15/p150, Vps14, and Vps30/Atg6 (Beclin-1 in mammals), Vps34 produces phosphatidylinositol 3-phosphate (PI3-P), a lipid that starts the nucleation event in the formation of the autophagosome [69].

Phagophore elongation, a crucial step in autophagosome formation, involves the action of two Ub-like conjugation systems. The first promotes the conjugation of the phosphatidylethanolamine (PE) molecule to the C terminus of Atg8-family proteins (Atg8/LC3-II/GABARAP). These small Ub-like proteins are synthesized as precursors that are cleaved at their C termini by the cysteine protease Atg4 [70]. The cleaved forms of these proteins (e.g., in the case of LC3, called LC3-I) are subsequently conjugated to PE by the consecutive actions of the Atg7 and Atg3 enzymes, thus producing the lipidated form (e.g., LC3-II). The lipidated form of Atg8 proteins is specifically associated with, and is required for the elongation of, the autophagosomal membrane [71]. In the parallel conjugation reaction, the Atg7 and Atg10 enzymes mediate covalent attachment of Atg12 to Atg5 [72]. The Atg5–Atg12 conjugate in a complex with Atg16 assists in the lipidation of Atg8 proteins and the extension of the phagophore [73,74]. Following the completion of the autophagic vesicle, its maturation involves fusion with an endosome (thus forming the amphisome) or directly with a lysosome, ultimately leading to the formation of the degradative organelle, the autolysosome.

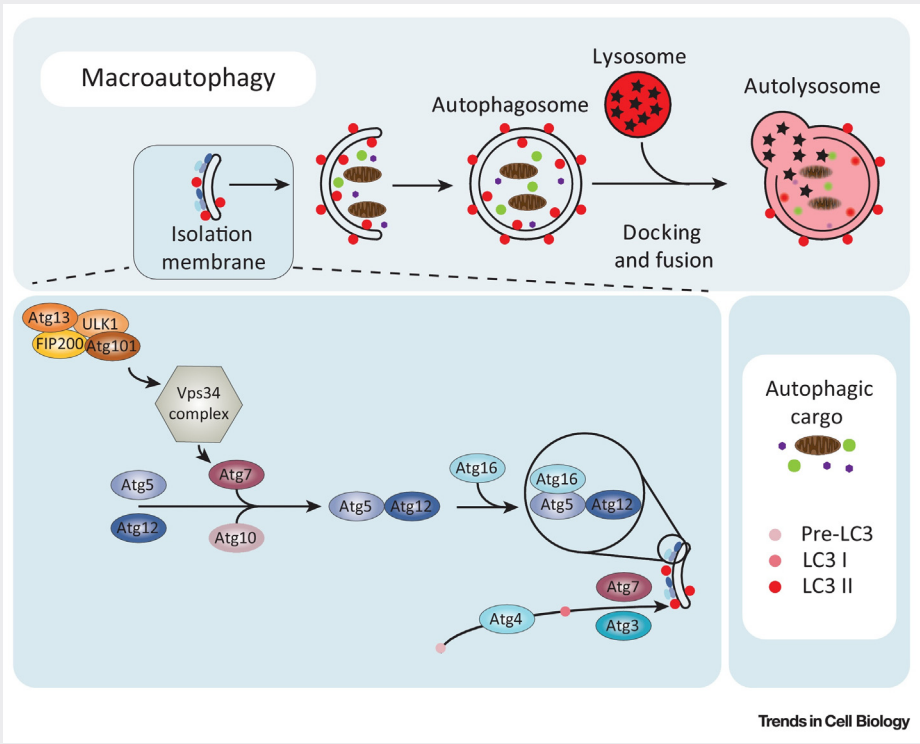

Figure I. The Core Machinery of (Macro)Autophagy.

(clearance of aggregate-prone proteins), mitophagy (dysfunctional mitochondria), and nucleophagy (damaged nuclear components) [13].

Double-Strand Break (DSB) Repair

DSBs are highly toxic lesions where both strands of DNA are broken. They have been shown to cause genomic instability and cell death. To protect against this, organisms have evolved highly regulated DDR mechanisms for sensing of the damage (Box 2) and activation of DSB repair

pathways (Figure 1). Defects in these processes have been shown to lead to numerous diseases as well as to drive cancer development [14].

There are two main pathways responsible for the repair of DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR) (Figure 1). NHEJ is active throughout the whole of the cell cycle whereas HR is active only during the S and G_2 phases when sister chromatids are available as templates for repair. The use of a homologous template by HR allows error-free repair [15–23]. NHEJ, by contrast, relies on the ligation of DNA ends without the use of a template, resulting in an increased occurrence of erroneous repair products [24]. Inhibition or absence of HR leads to reliance on the error-prone NHEJ pathway, leading to increased mutations and chromosomal rearrangements. Key components of the HR pathway such BRCA2 and RAD51 are known tumor suppressors, with inactivating mutations being linked to genomic instability [25,26]. Besides DSB repair, HR factors are important for the protection of stalled replication forks in a recombination-independent manner [27]. RAD51 is recruited to stalled replication forks through an Mre11- and BRCA2-dependent mechanism. In a tightly balanced process, Mre11 is thought to extend the single-stranded DNA (ssDNA) gap left by the replisome and to facilitate post-replication repair while BRCA2 leads to RAD51 loading, which protects arrested forks by preventing overextension of ssDNA gaps [27].

By contrast, NHEJ does not require a homologous template to mediate DSB repair. Instead, DNA ends are directly joined by DNA ligases (Figure 1) [24,28–33]. NHEJ is not only required to repair DSBs generated by exogenous and endogenous stress; it is also needed for the ligation of breaks generated during V(D)J recombination, a process required for immunoglobulin and T cell receptor (TCR) diversity [34]. Immunoglobulin and TCR genes contain variable (V), diversity (D), and joining (J) sections that are joined by the NHEJ machinery giving rise to mature V(D)J exons in B and T cells, respectively [35]. DSBs are generated between recombination signal sequences (RSSs) and coding DNA by the RAG1 and RAG2 proteins [36]. The resulting DSBs have blunt

Box 2. The DDR

DSBs, as well as ssDNA, lead to potent activation of the DDR (Figure I). Specialized sensing complexes are responsible for the detection of DNA lesions and initiation of the DDR. Initially, DSBs are sensed and processed by the Mre11 complex (MRN) comprisingf Mre11/Rad50/Nbs1, while ataxia telangiectasia and Rad3 related (ATR), ATR-interacting protein (ATRIP), replication protein A (RPA), Rad9/Rad1/Hus1, and Rad17/RSR are all involved in sensing ssDNA. Following detection, sensors recruit phosphoinositide kinase-related kinases (PIKKs), ATR, and ataxia telangiectasia mutated (ATM) to the site of damage leading to the local phosphorylation of histone H2A.X (H2A.X) to γH2A.X [75].

At DSBs the ATM-mediated phosphorylation of H2A.X leads to further recruitment of ATM, setting up a positive feedback loop that results in the spread of γH2A.X up to 2 Mb from the site of damage [76]. This amplification step is further facilitated by the DDR mediators mediator of DNA damage checkpoint 1 (MDC1) and p53-binding protein1 (53BP1) [77] and establishes a platform to which other DNA repair factors are recruited. The recruitment of 53BP1 and BRCA1 plays an important role in DNA repair pathway choice. BRCA1 has been shown to promote the removal of 53BP1 during S phase, promoting DNA resection and repair through the HR pathway [78]. Conversely, 53BP1 has been shown to negatively regulate resection in G_1 [79].

At SSBs stranded DNA is bound by RPA, signaling the recruitment of ATR [80]. ATR activity is further amplified by the heterotrimeric 9–1–1 complex (RAD9, RAD1, and HUS1) and topoisomerase-II-binding protein 1 (TOPBP1) [81,82]. Downstream of TOPBP Claspin leads to the activation of CHK1 [83]. Both CHK1 and a related protein kinase, CHK2, are phosphorylated downstream of ATM and ATR activation leading to their increased nuclear diffusion and the spread of DDR signaling [84]. DSBs favor activation of ATM/CHK2 and SSBs favor activation of ATR/CHK1. However, the processing of DSBs during the S or G_2 phase of the cell cycle can result in the generation of ssDNA and ATR activation [85].

Ultimately, activation of the DDR leads to the enforcement of cell-cycle checkpoints through multiple signaling pathways such as p53 and cell-division cycle 25 (CDC25) phosphatase. The primary objective of this cell-cycle arrest is to allow the proper processing and repair of DNA damage or activation of apoptosis and cellular senescence (reviewed in [86]).

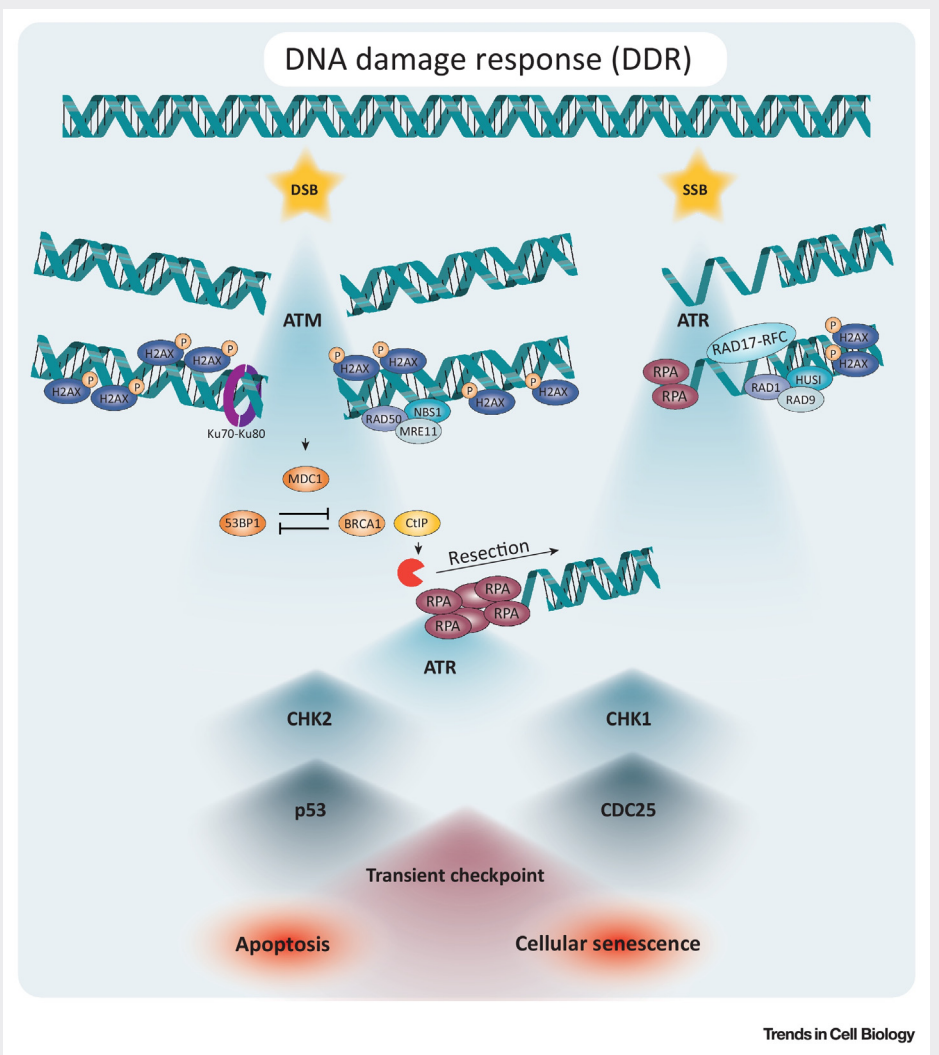

Figure I. The DNA Damage Response.

DNA ends with a hairpin structure that requires processing by Artemis before ligation via NHEJ [37,38]. Mutations in NHEJ genes can lead to severe combined immunodeficiency (SCID) due to an inability to properly complete the V(D)J [39]. Consistent with its requirement in NEHJ, cells lacking Artemis show considerable radiosensitivity and significant chromosomal abnormalities [40–42].

Autophagy and Genomic Integrity

Evidence for the role of autophagy in genome maintenance was first observed in autophagy-deficient (*beclin1*$^{+/-}$) tumor cells, where lack of autophagy results in increased markers of genomic instability such as DNA damage, gene amplification, and aneuploidy [43]. This has been attributed to increased levels of ROS and p62, as reduction of either alleviates the increased damage seen in autophagically compromised cells [44]. Autophagy has been shown to reduce levels of ROS though the elimination of damaged mitochondria, a process called mitophagy [45]. It has also been shown that p62-dependent selective degradation of KEAP1 by autophagy leads to the release of Nrf2 and activation of antioxidant defenses [46]. It is therefore possible that

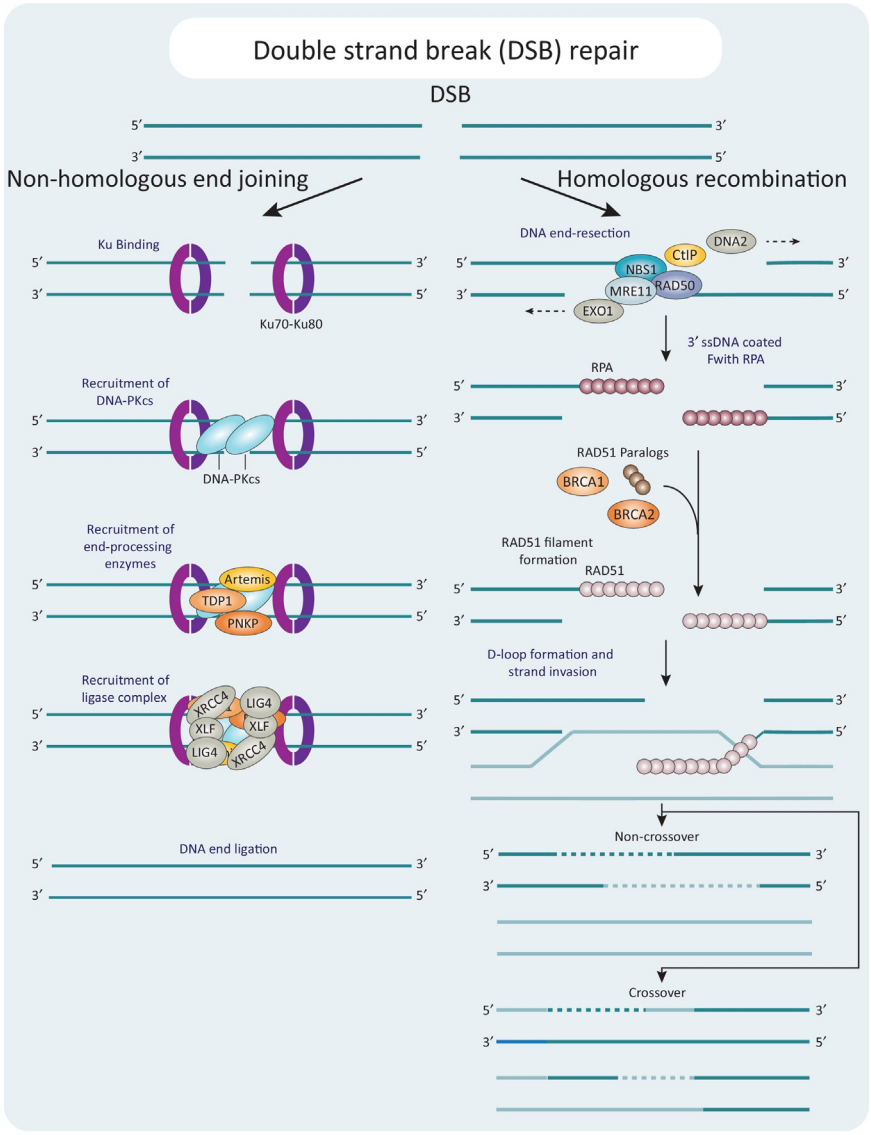

Figure 1. Double-Strand Break (DSB) Repair. Non-homologous end joining (NHEJ) and homologous recombination (HR) represent the two main DSB repair pathways. NHEJ is initiated by the recruitment and binding of the Ku70/80 heterodimer to exposed DNA ends. This forms a ring-like structure that encircles the spiral structure of the DNA and holds the DNA ends in the correct phase to facilitate end joining. Following this, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is recruited and activated to bind and stabilize the DNA ends, keeping them in close proximity. Next, end-processing factors such as tyrosyl-DNA phosphodiesterase 1 (TDP1), polynucleotide kinase/phosphatase (PNKP), Artemis, and AP endonuclease 1 (APE1) are recruited and prepare the DNA ends for ligation by the X-ray repair cross-complementing protein 4 (XRCC4)–XRCC4-like factor (XLF)–DNA ligase 4 (LIG4) complex. HR is initiated by the formation of 3′ single-stranded DNA (ssDNA) stretches that result from DNA-end resection. This is a tightly regulated process requiring the activity of several factors such as the MRN complex, CtIP, exonuclease 1 (EXO1), DNA replication ATP-dependent helicase (DNA2), and Bloom's syndrome helicase (BLM). Following resection ssDNA is bound by replication protein A (RPA). Next, RPA is removed and replaced with RAD51 to form a RAD51–ssDNA nucleofilament, a key step in HR. The formation of this filament is mediated by BRCA1, BRCA2, and RAD51 paralogs; however, the exact mechanisms remain not fully understood. This RAD51–ssDNA nucleofilament then undergoes homology search, strand invasion, and displacement D-loop formation. Next, RAD51 is removed by helicases such as HELQ and RAD54 allowing access by DNA polymerases such as DNA polymerase delta, POLN, and eta, leading to extension of the D loop. Finally, these HR structures are processed leading to the resolution of the DSB.

inhibition of autophagy can lead to genomic instability due to high levels of genotoxic ROS. However, recent studies also suggest that autophagy may impact genome integrity through the degradation of nuclear components and modulation of DSB repair pathways.

Degradation of Nuclear Components by Autophagy

In mammalian cells autophagy has been largely implicated in the turnover of cytoplasmic proteins and organelles; however, degradation of nuclear components has also been observed. In very specific cases, autophagy-mediated degradation of whole nuclei has been observed in lower organisms such as the fungus *Aspergillus oryzae* during syncytium formation and in the binuclear unicellular organism *Tetrahymena thermophila* to eliminate one of the two nuclei [47,48]. In most cellular contexts, bulk nuclear degradation via autophagy would be expected to lead to loss of genomic material, which would result in lethality. Instead, a growing body of evidence suggests a tightly controlled, selective autophagic turnover of nuclear components in mammalian cells. Cells with nuclear envelope defects (envelopathies) caused by mutations in proteins such as lamin A and emerin harbor autolysosomes containing nuclear components. Similar autolysosomes were observed in wild-type cells, albeit at a much lower frequency, suggesting that this process is not solely confined to nuclear envelopathies [49]. Furthermore, autophagy has been shown to contribute to the removal of micronuclei resulting from improper chromosome segregation during cell-cycle perturbation [50]. Micronuclei that are positive for the DNA damage marker γH2A.X are targeted for autophagic degradation by the receptor protein p62 [50] (Figure 2). Interestingly, cells with an artificial aneuploid phenotype display increased autophagic activity as well as elevated levels of p62 [51]. Inhibition of autophagy in the context of envelopathies, cell-cycle perturbation, and artificial aneuploidy all resulted in increased DNA damage and genomic instability [52]. Taken together these data suggest that autophagy plays a protective role in the context of chromosome missegregation.

The autophagic degradation of cytoplasmic chromatin fragments (CCFs) was recently shown to occur in cells following induction of oncogene-induced senescence (OIS) and replicative senescence (RS) [53] (Figure 2). Here, lamin A/C-negative, γH2A.X- and H3K27me3-positive CCFs bud from the nucleus and are processed by autophagy through Ub-mediated interaction with p62. It is hypothesized that this process leads to the disruption of lamin B1 and histone loss associated with the stabilization of cellular senescence [53]. Mechanistically, lamin B1 and associated chromatin is shuttled from the nucleus to the cytoplasm and degraded by autophagy in response to oncogene activation. This process is mediated by the direct interaction of LC3 with lamin B1 within the nucleus (Figure 2) [54]. Disruption of the LC3–lamin B1 interaction or inhibition of autophagy prevents lamin B1 loss and attenuates OIS. These data suggest that autophagic degradation of nuclear components plays a role in genome maintenance through the stabilization of cellular senescence, acting as a barrier to tumorigenesis following oncogene activation [53,54].

Autophagy and DNA Repair

Recently, the role of autophagy in genome maintenance was further expanded with evidence suggesting a more direct role of autophagy in DNA damage repair.

The first of these studies noted that inhibition of histone deacetylases (HDACs) with valproic acid (VPA) treatment resulted in the activation of autophagy and increased autophagic degradation of the DNA endonuclease Sae2 in yeast [55]. This was shown to result in decreased levels of end resection and decreased cell survival in response to genotoxic agents. Furthermore, knockout of Rpd3 and Hda1 in yeast recapitulates VPA-induced phenotypes and this could be alleviated by knockout of the histone acetyltransferase (HAT) Gcn5, suggesting that they are involved in the regulation of Sae2 acetylation in response to DNA damage. Activation of autophagy with rapamycin treatment was also shown to reduce levels of Sae2, suggesting that the acetylation

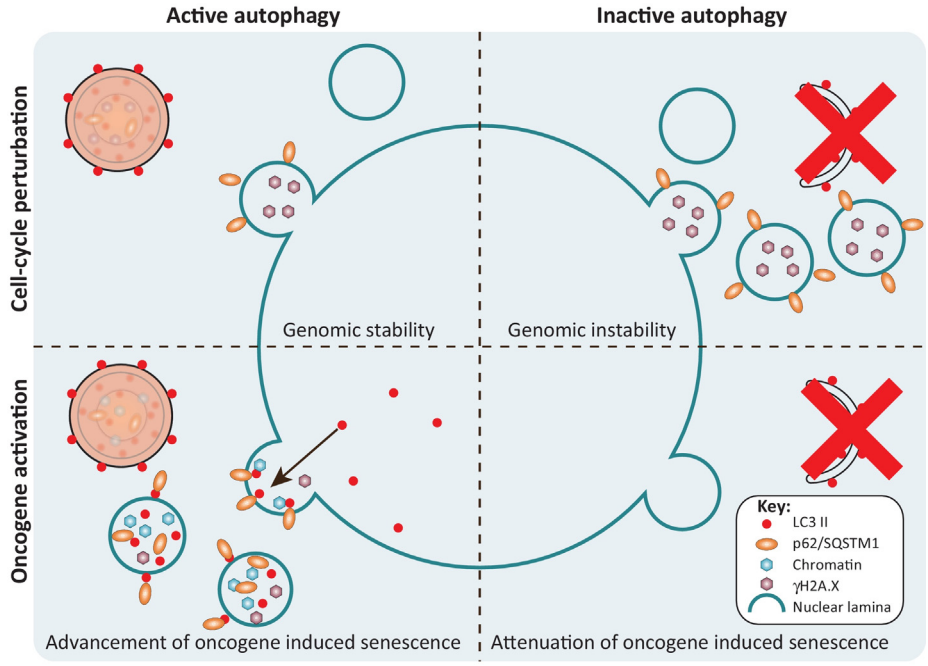

Figure 2. Autophagic Degradation of Nuclear Components. (A) Perturbation of the cell cycle leads to increased formation of micronuclei. These micronuclei are positive for the DNA damage marker γH2A.X and have been shown to be a target for autophagic degradation mediated by interaction with p62 and LC3. It is suggested that autophagy may help maintain genomic integrity via the removal of micronuclei, as its suppression results in accumulation of γH2A.X-positive micronuclei and genomic instability. (B) On oncogene activation autophagy is activated and mediates the degradation of proteins in the nuclear lamina as well as chromatin. LC3 present in the nucleus interacts with lamin B1 resulting in the shuttling of nuclear lamina proteins as well as associated chromatin from the nucleus to the cytoplasm. Once in the cytoplasm, LC3 and associated proteins are targeted for autophagic degradation by the autophagy receptor p62. This loss of nuclear lamina protein and chromatin leads to cell-cycle arrest and cellular senescence. Inhibition of autophagy prevents the oncogene-induced loss of chromatin and nuclear lamina proteins and attenuates oncogene-induced senescence. It has therefore been suggested that autophagy acts as a safeguard against oncogene-induced tumorigenesis.

and subsequent autophagic degradation of Sae2 may be a novel mechanism linking DNA repair and autophagy. Other studies propose that rapamycin treatment might suppress DSB repair [56]. However, it should be noted that inhibition of mammalian target of rapamycin (mTOR) by rapamycin impacts various cellular processes, such as protein synthesis and cell-cycle progression as well as apoptosis, outside its regulatory role in autophagy [57].

While some findings suggest that activation of autophagy can lead to impairment of DNA damage repair [55], several studies propose that inactivation of autophagy can also give rise to reduced DNA damage repair capacity [41,58–61]. For example, loss of autophagy through inducible knockout of *Atg7* leads to impaired DSB repair via the HR pathway. This defect was proposed to be due to enhanced proteasomal activity, which in turn enhances degradation of the HR mediator checkpoint kinase 1 (CHK1) [59] (Figure 3A). The two major DSB repair pathways can compensate for each another when one pathway is impaired [24]. Thus, cells lacking *Atg7* are hyper-reliant on NHEJ for DSB repair and inhibition of NHEJ caused rapid cell death of autophagy-deficient cells. This synthetic lethal relationship between autophagy and NHEJ presents an exciting therapeutic avenue by which to target autophagy-deficient cells, which are a common feature of human age-related diseases [62].

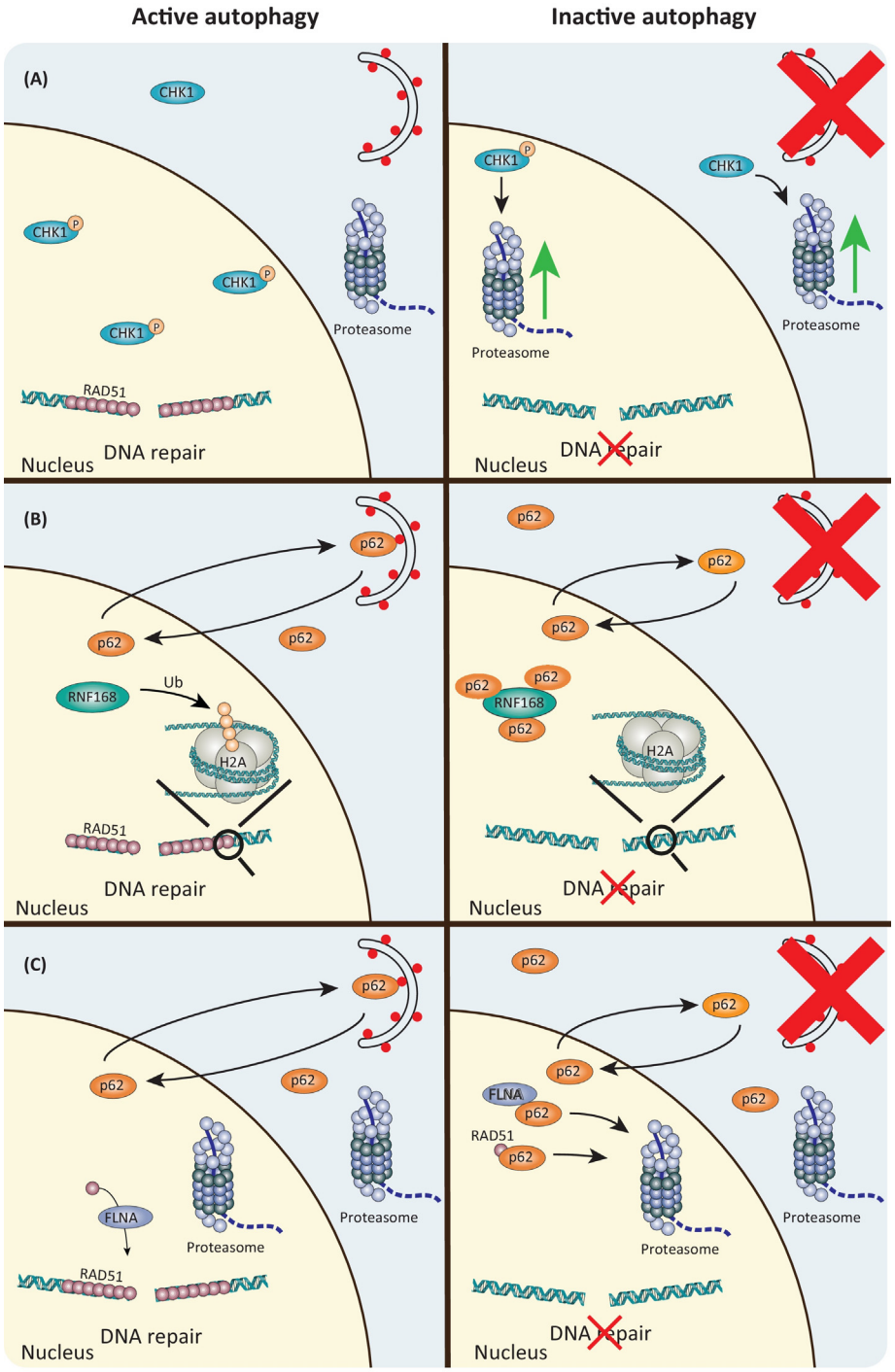

Figure 3. Mechanisms of Crosstalk between Autophagy and Double-Strand Break (DSB) Repair. (A) Loss/inhibition of autophagy leads to an increase in the proteasomal degradation of checkpoint kinase 1 (CHK1) resulting in impairment of repair via the homologous recombination (HR) pathway. This gives rise to hyperdependence on non-homologous end joining (NHEJ), genomic instability, and increased formation of micronuclei. (B) Accumulated nuclear p62 arising from loss/inhibition of autophagy binds to RNF168 and inhibits its ubiquitin ligase activity resulting in reduced recruitment of DNA repair factors and inhibition of DNA repair. (C) Accumulated nuclear p62 arising from loss/inhibition of autophagy targets FLNA and RAD51 for degradation via the proteasome resulting in reduced HR activity.

Furthermore, inhibition of autophagy by the genetic knockout of 200-kDa FAK-family-interacting protein (FIP200), a ULK1-interacting protein essential for autophagosome formation (Box 1), suppressed DNA damage repair and decreased cell viability following ionizing radiation and camptothecin treatment. Although the exact molecular mechanisms were not explored, knockdown of p62 alleviated the defect in repair and increased cell survival [58]. The latter result suggests that increased levels of p62 due to autophagy inhibition could be responsible for reduced DNA damage repair.

The observation of nuclear–cytoplasmic shuttling of p62 [63] provides an exciting link between the cytoplasmic process of autophagy and the nucleus. Both a nuclear export signal (NES) and two nuclear localization signals (NLSs) have been identified [63]. Two recent studies suggest that DNA repair is influenced by p62 specifically located in the nucleus, albeit through slightly differing models [61,64]. In one of the studies [64], increased levels of p62 resulting from inhibition of autophagy led to a decrease in DNA damage-induced chromatin ubiquitination. Mechanistically, this phenotype was explained by the interaction of p62 with RNF168 via the LIM-binding (LB) domain of p62 and MU1 domain of RNF168. This interaction inhibited the E3 ligase activity of RNF168 resulting in impaired chromatin ubiquitination and reduced recruitment of DNA repair proteins following DNA damage (Figure 3B). Similar to previous reports, inhibition of autophagy specifically inhibited HR-directed DSB repair, with cells showing decreased recruitment of HR proteins such as RAD51, RAP80, and BRCA1, while NHEJ remained unaffected [59,64]. CHK1 levels, however, remained unchanged, suggesting a mechanism distinct from that proposed previously [59]. Finally, using xenograft experiments overexpression of nuclear-export mutant p62(K7A/D69A/I314E) led to decreased tumor cell survival following irradiation. This phenotype was dependent on p62–RNF168 interaction as deletion of the LB domain of p62 abolished this affect [64]. It should be noted that RNF168 acts upstream of both HR and NHEJ and p62-mediated inhibition of chromatin ubiquitination leads to the perturbation of both pathways while autophagy inhibition affects HR only. Further work is required to understand this apparent contradiction.

An alternative mechanism for p62-dependent autophagy-mediated modulation of DNA repair has been reported [61]. Here, p62 was shown to inhibit HR-directed DSB repair through proteasomal degradation of filamin A (FLNA) and RAD51 in the nucleus [61] (Figure 3C). FLNA has previously been implicated in DNA repair, specifically through interaction with BRCA1/2 and recruitment of RAD51 in the HR repair pathway [65–67]. Similar to Wang *et al.* [64], the effect of autophagy on DNA repair appeared to be independent of CHK1 suggesting a mechanism distinct from that previously reported [59]. It is important, however, to note that this was observed following low levels of damage induction; therefore, changes in downstream signaling such as CHK1 may be less apparent. Finally, it was suggested that p62-mediated inhibition of DNA repair may be a driver of organismal aging that can be reversed by dietary restriction, a potent activator of autophagy [61].

In summary, autophagy has been shown to be directly involved in DSB repair [41,58–61]. Specifically, impairment of autophagy can inhibit repair through the HR pathway [59,61,64] either by increased proteasomal degradation of the HR-mediating protein CHK1 [59] or by increased levels of p62 [58,61,64], specifically in the nucleus [61,64]. Nuclear p62 has been shown to impact HR through the inhibition of RNF168-mediated chromatin ubiquitination [64] as well as by targeting the HR proteins RAD51 and FLNA for degradation via the proteasome [61]. Further work is required to understand the interplay between these mechanisms to clarify whether they operate separately or in cooperation.

Concluding Remarks

It is becoming increasingly clear that, despite being a cytoplasmic process, autophagy plays a key role in maintaining genomic stability. A range of mechanisms appear to be involved where autophagy can impact DNA maintenance indirectly (e.g., via modulation of ROS through processes such as mitophagy and antioxidant defense) as well as directly (e.g., through selective degradation of nuclear components and modulation of DSB repair). However, these initial studies raise several important questions that need to be addressed to move this area of research forward (see Outstanding Questions). As our understanding of the interplay between autophagy and genome maintenance and its impact on physiology expands, it will potentially present exciting new therapeutic opportunities.

Outstanding Questions

Besides lamin B1, what are the other substrates of LC3-mediated nuclear autophagy?

What are the nuclear interactors of p62 in response to DNA damage in addition to the already-identified RNF168, RAD51, and FLNA?

Besides DNA damage repair (FLNA and RAD51) and neurodegeneration (Ataxin1Q84), what other cellular processes are impacted by p62-mediated proteasomal degradation in the nucleus?

Why does increased p62 and loss of RNF168-mediated chromatin ubiquitination lead to inhibition of NHEJ in wild-type but not autophagy-deficient cells?

Does decreased repair capacity resulting from impaired autophagy contribute to organismal aging and age-related diseases, specifically cancer progression?

Can synthetic lethality between autophagy and NHEJ be exploited as a therapeutic target in diseases including cancer?

Acknowledgments

G.H. and V.I.K are funded by the BBSRC. V.I.K is funded by the NIHR Newcastle Biomedical Research Centre, the Newcastle upon Tyne Hospitals NHS Charity, and the British Skin Foundation.

References

1. Saul, R.L. and Ames, B.N. (1986) Background levels of DNA damage in the population. *Basic Life Sci.* 38, 529–535
2. Varshavsky, A. (1997) The ubiquitin system. *Trends Biochem. Sci.* 22, 383–387
3. Mijaljica, D. *et al.* (2011) Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. *Autophagy* 7, 673–682
4. Cuervo, A.M. and Wong, E. (2014) Chaperone-mediated autophagy: roles in disease and aging. *Cell Res.* 24, 92–104
5. Kaur, J. and Debnath, J. (2015) Autophagy at the crossroads of catabolism and anabolism. *Nat. Rev. Mol. Cell Biol.* 16, 461–472
6. Kopito, R.R. (2000) Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* 10, 524–530
7. Liu, Y. and Levine, B. (2015) Autosis and autophagic cell death: the dark side of autophagy. *Cell Death Differ.* 22, 367–376
8. Liu, Y. *et al.* (2013) Autosis is a Na^+,K^+-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia–ischemia. *Proc. Natl Acad. Sci. U. S. A.* 110, 20364–20371
9. Carlsson, S.R. and Simonsen, A. (2015) Membrane dynamics in autophagosome biogenesis. *J. Cell Sci.* 128, 193–205
10. van der Vaart, A. *et al.* (2008) A picky eater: exploring the mechanisms of selective autophagy in human pathologies. *Traffic* 9, 281–289
11. Komatsu, M. *et al.* (2007) Constitutive autophagy: vital role in clearance of unfavorable proteins in neurons. *Cell Death Differ.* 14, 887–894
12. Birgisdottir, Å.B. *et al.* (2013) The LIR motif – crucial for selective autophagy. *J. Cell Sci.* 126, 3237–3247
13. Zaffagnini, G. and Martens, S. (2016) Mechanisms of selective autophagy. *J. Mol. Biol.* 428, 1714–1724
14. Jackson, S.P. and Bartek, J. (2009) The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078
15. Li, X. *et al.* (2009) PCNA is required for initiation of recombination-associated DNA synthesis by DNA polymerase delta. *Mol. Cell* 36, 704–713
16. McIlwraith, M.J. *et al.* (2005) Human DNA polymerase eta promotes DNA synthesis from strand invasion intermediates of homologous recombination. *Mol. Cell* 20, 783–792
17. Moldovan, G.L. *et al.* (2010) DNA polymerase POLN participates in cross-link repair and homologous recombination. *Mol. Cell. Biol.* 30, 1088–1096
18. Nakamura, K. *et al.* (2010) Collaborative action of Brca1 and CtIP in elimination of covalent modifications from double-strand breaks to facilitate subsequent break repair. *PLoS Genet.* 6, e1000828
19. San Filippo, J. *et al.* (2008) Mechanism of eukaryotic homologous recombination. *Annu. Rev. Biochem.* 77, 229–257
20. Solinger, J.A. *et al.* (2002) Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. *Mol. Cell* 10, 1175–1188
21. Ward, J.D. *et al.* (2010) Overlapping mechanisms promote postsynaptic RAD-51 filament disassembly during meiotic double-strand break repair. *Mol. Cell* 37, 259–272
22. Yang, H. *et al.* (2005) The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA–ssDNA junction. *Nature* 433, 653–657
23. Zhu, Z. *et al.* (2008) Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134, 981–994
24. Chapman, J.R. *et al.* (2012) Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* 47, 497–510
25. Golmard, L. *et al.* (2013) Germline mutation in the *RAD51B* gene confers predisposition to breast cancer. *BMC Cancer* 13, 484
26. Wooster, R. *et al.* (1995) Identification of the breast cancer susceptibility gene *BRCA2*. *Nature* 378, 789–792
27. Costanzo, V. (2011) Brca2, Rad51 and Mre11: performing balancing acts on replication forks. *DNA Repair* 10, 1060–1065
28. Ahnesorg, P. *et al.* (2006) XLF interacts with the XRCC4–DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* 124, 301–313
29. Meek, K. *et al.* (2007) *Trans* autophosphorylation at DNA-dependent protein kinase's two major autophosphorylation site clusters facilitates end processing but not end joining. *Mol. Cell. Biol.* 27, 3881–3890
30. Moshous, D. *et al.* (2001) Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 105, 177–186
31. Pommier, Y. *et al.* (2014) Tyrosyl-DNA-phosphodiesterases (TDP1 and TDP2). *DNA Repair (Amst.)* 19, 114–129
32. Walker, J.R. *et al.* (2001) Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412, 607–614
33. Weterings, E. and van Gent, D.C. (2004) The mechanism of non-homologous end-joining: a synopsis of synapsis. *DNA Repair (Amst.)* 3, 1425–1435
34. Jung, D. *et al.* (2006) Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu. Rev. Immunol.* 24, 541–570
35. Schatz, D.G. (2004) V(D)J recombination. *Immunol. Rev.* 200, 5–11
36. McBlane, J.F. *et al.* (1995) Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. *Cell* 83, 387–395
37. van Gent, D.C. *et al.* (1996) The RAG1 and RAG2 proteins establish the 12/23 rule in V(D)J recombination. *Cell* 85, 107–113
38. Ma, Y. *et al.* (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 108, 781–794
39. Woodbine, L. *et al.* (2014) The clinical impact of deficiency in DNA non-homologous end-joining. *DNA Repair (Amst.)* 16, 84–96

40. Riballo, E. *et al.* (2004) A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to γ-H2AX foci. *Mol. Cell* 16, 715–724
41. Wang, J. *et al.* (2005) Artemis deficiency confers a DNA double-strand break repair defect and Artemis phosphorylation status is altered by DNA damage and cell cycle progression. *DNA Repair (Amst.)* 4, 556–570
42. Rooney, S. *et al.* (2002) Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. *Mol. Cell* 10, 1379–1390
43. Karantza-Wadsworth, V. *et al.* (2007) Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev.* 21, 1621–1635
44. Mathew, R. *et al.* (2009) Autophagy suppresses tumorigenesis through elimination of p62. *Cell* 137, 1062–1075
45. Kurihara, Y. *et al.* (2012) Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation of mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. *J. Biol. Chem.* 287, 3265–3272
46. Komatsu, M. *et al.* (2010) The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat. Cell Biol.* 12, 213–223
47. Shoji, J.-Y. *et al.* (2010) Macroautophagy-mediated degradation of whole nuclei in the filamentous fungus *Aspergillus oryzae*. *PLoS One* 5, e15650
48. Lu, E. and Wolfe, J. (2001) Lysosomal enzymes in the macronucleus of *Tetrahymena* during its apoptosis-like degradation. *Cell Death Differ.* 8, 289–297
49. Park, Y.E. *et al.* (2009) Autophagic degradation of nuclear components in mammalian cells. *Autophagy* 5, 795–804
50. Rello-Varona, S. *et al.* (2012) Autophagic removal of micronuclei. *Cell Cycle* 11, 170–176
51. Stingele, S. *et al.* (2012) Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol. Syst. Biol.* 8, 608
52. Ariyoshi, K. *et al.* (2016) Induction of genomic instability and activation of autophagy in artificial human aneuploid cells. *Mutat. Res.* 790, 19–30
53. Ivanov, A. *et al.* (2013) Lysosome-mediated processing of chromatin in senescence. *J. Cell Biol.* 202, 129–143
54. Dou, Z. *et al.* (2015) Autophagy mediates degradation of nuclear lamina. *Nature* 527, 105–109
55. Robert, T. *et al.* (2011) HDACs link the DNA damage response, processing of double-strand breaks and autophagy. *Nature* 471, 74–79
56. Chen, H. *et al.* (2011) The mTOR inhibitor rapamycin suppresses DNA double-strand break repair. *Radiat. Res.* 175, 214–224
57. Shimobayashi, M. and Hall, M.N. (2014) Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat. Rev. Mol. Cell Biol.* 15, 155–162
58. Bae, H. and Guan, J.-L. (2011) Suppression of autophagy by FIP200 deletion impairs DNA damage repair and increases cell death upon treatments with anti-cancer agents. *Mol. Cancer Res.* 9, 1232–1241
59. Liu, E.Y. *et al.* (2015) Loss of autophagy causes a synthetic lethal deficiency in DNA repair. *Proc. Natl Acad. Sci. U.S.A.* 112, 773–778
60. Lin, W. *et al.* (2015) Autophagy confers DNA damage repair pathways to protect the hematopoietic system from nuclear radiation injury. *Sci. Rep.* 5, 12362
61. Hewitt, G. *et al.* (2016) SQSTM1/p62 mediates crosstalk between autophagy and the UPS in DNA repair. *Autophagy* 12, 1917–1930
62. Jiang, P. and Mizushima, N. (2014) Autophagy and human diseases. *Cell Res.* 24, 69–79
63. Pankiv, S. *et al.* (2010) Nucleocytoplasmic shuttling of p62/SQSTM1 and its role in recruitment of nuclear polyubiquitinated proteins to promyelocytic leukemia bodies. *J. Biol. Chem.* 285, 5941–5953
64. Wang, Y. *et al.* (2016) Autophagy regulates chromatin ubiquitination in DNA damage response through elimination of SQSTM1/p62. *Mol. Cell* 63, 34–48
65. Velkova, A. *et al.* (2010) Identification of filamin A as a BRCA1-interacting protein required for efficient DNA repair. *Cell Cycle* 9, 1421–1433
66. Yue, J. *et al.* (2012) Filamin-A as a marker and target for DNA damage based cancer therapy. *DNA Repair (Amst.)* 11, 192–200
67. Yue, J. *et al.* (2009) The cytoskeleton protein filamin-A is required for an efficient recombinational DNA double strand break repair. *Cancer Res.* 69, 7978–7985
68. Mizushima, N. and Komatsu, M. (2011) Autophagy: renovation of cells and tissues. *Cell* 147, 728–741
69. Kang, R. *et al.* (2011) The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ.* 18, 571–580
70. Kirisako, T. *et al.* (2000) The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J. Cell Biol.* 151, 263–276
71. Ichimura, Y. *et al.* (2000) A ubiquitin-like system mediates protein lipidation. *Nature* 408, 488–492
72. Mizushima, N. *et al.* (1998) A protein conjugation system essential for autophagy. *Nature* 395, 395–398
73. Kuma, A. *et al.* (2002) Formation of the approximately 350-kDa Apg12–Apg5.Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. *J. Biol. Chem.* 277, 18619–18625
74. Mizushima, N. *et al.* (1999) Apg16p is required for the function of the Apg12p–Apg5p conjugate in the yeast autophagy pathway. *EMBO J.* 18, 3888–3896
75. Shiloh, Y. (2006) The ATM-mediated DNA-damage response: taking shape. *Trends Biochem. Sci.* 31, 402–410
76. Bewersdorf, J.R. *et al.* (2006) H2AX chromatin structures and their response to DNA damage revealed by 4Pi microscopy. *Proc. Natl Acad. Sci. U. S. A.* 103, 18137–18142
77. Wang, B. *et al.* (2002) 53BP1, a mediator of the DNA damage checkpoint. *Science* 298, 1435–1438
78. Bunting, S.F. *et al.* (2010) 53BP1 inhibits homologous recombination in *Brca1*-deficient cells by blocking resection of DNA breaks. *Cell* 141, 243–254
79. Bothmer, A. *et al.* (2010) 53BP1 regulates DNA resection and the choice between classical and alternative end joining during class switch recombination. *J. Exp. Med.* 207, 855–865
80. Cortez, D. *et al.* (2001) ATR and ATRIP: partners in checkpoint signaling. *Science* 294, 1713–1716
81. Weiss, R.S. *et al.* (2002) Hus1 acts upstream of Chk1 in a mammalian DNA damage response pathway. *Curr. Biol.* 12, 73–77
82. Kumagai, A. *et al.* (2006) TopBP1 activates the ATR–ATRIP complex. *Cell* 124, 943–955
83. Liu, S. *et al.* (2006) Claspin operates downstream of TopBP1 to direct ATR signaling towards Chk1 activation. *Mol. Cell. Biol.* 26, 6056–6064
84. Bekker-Jensen, S. *et al.* (2006) Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. *J. Cell Biol.* 173, 195–206
85. Jazayeri, A. *et al.* (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat. Cell Biol.* 8, 37–45
86. Roos, W.P. *et al.* (2016) DNA damage and the balance between survival and death in cancer biology. *Nat. Rev. Cancer* 16, 20–33