

## Nuclear DNA damage signalling to mitochondria in ageing

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**Abstract** | Mitochondrial dysfunction is a hallmark of ageing, and mitochondrial maintenance may lead to increased healthspan. Emerging evidence suggests a crucial role for signalling from the nucleus to mitochondria (NM signalling) in regulating mitochondrial function and ageing. An important initiator of NM signalling is nuclear DNA damage, which accumulates with age and may contribute to the development of age-associated diseases. DNA damage-dependent NM signalling constitutes a network that includes nuclear sirtuins and controls genomic stability and mitochondrial integrity. Pharmacological modulation of NM signalling is a promising novel approach for the prevention and treatment of age-associated diseases.

Mitochondria are present in the cytoplasm of almost all mammalian cells. They provide energy by generating ATP and are crucial for many signalling pathways. Mitochondria constantly adjust their morphology and function in response to changes in the microenvironment, such as exposure to endogenous or exogenous stress<sup>1,2</sup>. Healthy mitochondria are maintained by multiple processes, including fusion and fission, DNA repair and mitophagy (clearance of damaged mitochondria)<sup>2–6</sup>. Impairment of any of these processes can lead to mitochondrial dysfunction and contribute to mitochondrial diseases, neurodegenerative disorders, cancer, diabetes, heart disease, immunodeficiency and ageing<sup>1,7–10</sup>. For example, impairment of mitophagy is implicated in Parkinson disease, Alzheimer disease and pathological ageing<sup>11–13</sup>. Although the aetiology of mitochondrial diseases has largely been attributed to defects in mitochondrial proteins, mutations in non-mitochondrial proteins or impairment of nucleus-to-mitochondria signalling (NM signalling) can also promote mitochondrial dysfunction. Thus, a better understanding of the molecular signalling mechanisms that influence mitochondrial functions is important in relation to human health.

Owing to its central function in energy metabolism, a healthy mitochondrial pool is crucial for organismal health. Indeed, some data suggest that damaged mitochondria accumulate with age in organisms spanning the evolutionary spectrum, from unicellular organisms to humans<sup>10,12,14–16</sup>. However, age-associated changes in gross mitochondrial function, such as oxidative phosphorylation, have not been consistently detected<sup>17,18</sup>. Thus, age-associated mitochondrial changes may not be the primary drivers, but rather a downstream consequence, of nuclear signalling pathways that drive the ageing process<sup>17,19,20</sup>.

Recent evidence suggests that compromised NM signalling is a key component of mammalian ageing. DNA damage is well known to affect DNA replication, transcription and many signalling pathways, but how this damage invokes signalling to mitochondria is a new area of investigation. Several DNA repair-deficient, premature ageing diseases, such as Cockayne syndrome, xeroderma pigmentosum group A (XPA) and ataxia telangiectasia, involve mitochondrial alterations consisting of increased mitochondrial membrane potential and increased oxygen consumption rates — traits that are indicative of adaptive responses<sup>3,12,21–23</sup>. In this Review, we focus on NM signalling pathways that are activated in response to genotoxic stimuli, especially DNA damage, and their roles in the maintenance of mitochondrial integrity and ageing (FIG. 1). We also discuss emerging findings suggesting that intervention in NM signalling can alleviate age-associated physiological changes. Importantly, NM signalling is closely coupled with signalling from mitochondria to the nucleus, including the mitochondrial unfolded protein response (UPR<sup>mt</sup>). Recent findings suggest that the nuclear DNA damage response regulates protein homeostasis in mitochondria through activation of the UPR<sup>mt</sup> (for more details about the molecular pathways involved, see REFS 16, 24–27).

### Nuclear DNA repair and mitochondrial health

Progressive damage to nuclear DNA and mitochondrial DNA (mtDNA) is considered to be a prominent contributing factor to the ageing process and may underlie several age-associated diseases<sup>4,20,28,29</sup>. Cells have multiple DNA repair pathways to rectify the myriad types of DNA lesions that occur and to maintain DNA integrity in the nucleus and in mitochondria (BOX 1).

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**Cockayne syndrome**

A rare accelerated-ageing disease with progressive neurodegeneration, caused by mutations in genes encoding two DNA repair proteins, CSA and CSB.

**Xeroderma pigmentosum**

A rare autosomal-recessive disorder characterized by severe sun sensitivity and skin cancer, associated with mutation of genes encoding a group of DNA repair proteins, XPA to XPG.

**Ataxia telangiectasia**

A genomic instability disease with progressive cerebellar neurodegeneration caused by mutation of the ataxia telangiectasia mutated (*ATM*) gene, encoding the kinase ATM, which is a master regulator of DNA damage processing.

**Preiss–Handler pathway**

A NAD<sup>+</sup> biosynthetic process that consumes dietary nicotinic acid.

**Salvage pathway**

A NAD<sup>+</sup> biosynthetic pathway that uses nicotinamide to generate nicotinamide mononucleotide (NMN), which is then transformed into NAD<sup>+</sup>.

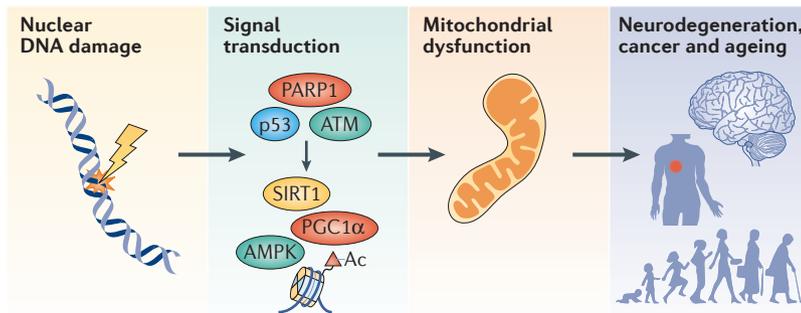
Key nuclear DNA repair pathways include DNA double-strand break repair (DSBR), base excision repair (BER) and nucleotide excision repair (NER). Multiple mitochondria-associated adaptive processes are regulated by nuclear transcription regulators, such as peroxisome proliferator-activated receptor- $\gamma$  co-activator 1 $\alpha$  (PGC1 $\alpha$ ), hypoxia inducible factor 1 $\alpha$  (HIF1 $\alpha$ ), the forkhead box O (FOXO) proteins and some sirtuin family members. Sirtuins are a group of NAD<sup>+</sup>-dependent deacetylases that can alter the activities of diverse proteins (BOX 2). Coordination between nuclear DNA repair proteins and nuclear sirtuins (SIRT1, SIRT6 and SIRT7) reveals a facet of how nuclear DNA repair proteins may regulate mitochondrial homeostasis (FIG. 2).

**The PARP1 and NAD<sup>+</sup>–SIRT1–PGC1 $\alpha$  axis.** A prominent pathway linking nuclear DNA damage to mitochondrial homeostasis is the NAD<sup>+</sup>–SIRT1–PGC1 $\alpha$  axis, in which NAD<sup>+</sup> has a pivotal role. NAD<sup>+</sup> is a rate-limiting metabolite for poly(ADP-ribose) polymerase 1 (PARP1) and SIRT1 activities, making the cellular content of NAD<sup>+</sup> an important index for cell fate and organismal health (see below). Intracellular NAD<sup>+</sup> levels are usually approximately 0.2–0.5 mM, depending on cell type and condition<sup>27</sup>. There is a subcellular difference in NAD<sup>+</sup> content between the nucleus and mitochondria, as the nuclear NAD<sup>+</sup> content is ~0.07 mM — much lower than in mitochondria, where it is 0.25 mM or greater<sup>27,30</sup>. The cellular NAD<sup>+</sup> level is tightly regulated by three biosynthetic pathways, the Preiss–Handler pathway, *de novo* biosynthesis and the salvage pathway<sup>27,31</sup>. There is an age-dependent decrease in NAD<sup>+</sup>, and NAD<sup>+</sup> is also reduced in several pathologies,

suggesting a contributory role for NAD<sup>+</sup> depletion in ageing and age-related diseases. The regulation of NAD<sup>+</sup> metabolism and its importance in health and ageing are discussed in two excellent recent reviews<sup>27,31</sup>.

Mammalian SIRT1 is thought to be involved in epigenetic regulation, mitochondrial maintenance and the ageing process. SIRT1, which is a prominent nuclear sirtuin, regulates various cellular pathways and may delay the progression of ageing through deacetylation of several substrates, including PGC1 $\alpha$ , which is a master regulator of mitochondrial biogenesis<sup>32</sup>. The deacetylation activity of SIRT1 and other sirtuins requires consumption of NAD<sup>+</sup>, which is a cofactor also involved in DNA damage repair<sup>12,33–35</sup>. When DNA breaks occur, the DNA break sensor PARP1 detects break sites and initiates DNA repair signalling through generation of PAR, a process called PARylation, which consumes NAD<sup>+</sup> (REF. 36) (FIG. 2). Interestingly, PARP1 and SIRT1 have comparable  $K_m$  values for NAD<sup>+</sup> (50–97  $\mu$ M and 94–96  $\mu$ M, respectively)<sup>27</sup>. PARylation promotes the rapid recruitment of PAR-binding DNA repair proteins to locate and efficiently repair the DNA damage, as well as promoting the decondensation of chromatin around the damage site. Aged and DNA repair-deficient laboratory animal models exhibited persistent hyperactivation of PARP1, lower SIRT1 activity and NAD<sup>+</sup> depletion. PARP1 inhibition or NAD<sup>+</sup> supplementation restored NAD<sup>+</sup> levels and SIRT1 activity<sup>12,16,27</sup>. Thus, PARP1 and SIRT1 can compete for NAD<sup>+</sup>, and increased consumption of NAD<sup>+</sup> by one of these proteins may limit the activity of the other (FIGS 2,3a). When cells with a very high energy requirement, such as neurons or cardiac myocytes, are subjected to severe reductions in energy availability — such as during an ischaemic event (stroke or myocardial infarction) — increased consumption of NAD<sup>+</sup> by PARP1 (REFS 37,38) and SIRT1 (REF. 39) may contribute to a cellular energy crisis that triggers cell death. The molecular details of the nature of the competition between PARP1 and SIRT1 for NAD<sup>+</sup> as a substrate merit further investigation.

Imbalances of the activities of PARP1 and SIRT1 have been associated with ageing and pathological processes. An age-dependent increase in PARylation was observed in various experimental animals (including nematodes and mice)<sup>12,16</sup>, and PARP activation has been detected in a wide range of human diseases, including chronic heart failure, stroke, Alzheimer disease and Parkinson disease<sup>40</sup>. Deletion of the *Parp1* gene increased NAD<sup>+</sup> levels, SIRT1 activity and mitochondrial biogenesis in mice<sup>41</sup>, and pharmacological inhibition of PARP1 increased mitochondrial biogenesis and extended lifespan in wild-type nematodes<sup>16</sup>. Compromised balance between the activities of PARP1 and SIRT1 signalling may also contribute to premature ageing syndromes, such as in XPA and Cockayne syndrome. Individuals with XPA are UV-sensitive, with a high risk of skin cancers, and they suffer from neurodegeneration, including progressive cerebral and cerebellar atrophy, neuropathy and sensorineural hearing loss<sup>12,42</sup>. XPA is caused by mutation of the gene encoding the protein XPA, which participates in NER<sup>43</sup>. Patients with Cockayne syndrome show photosensitivity (but no cancer susceptibility), with major

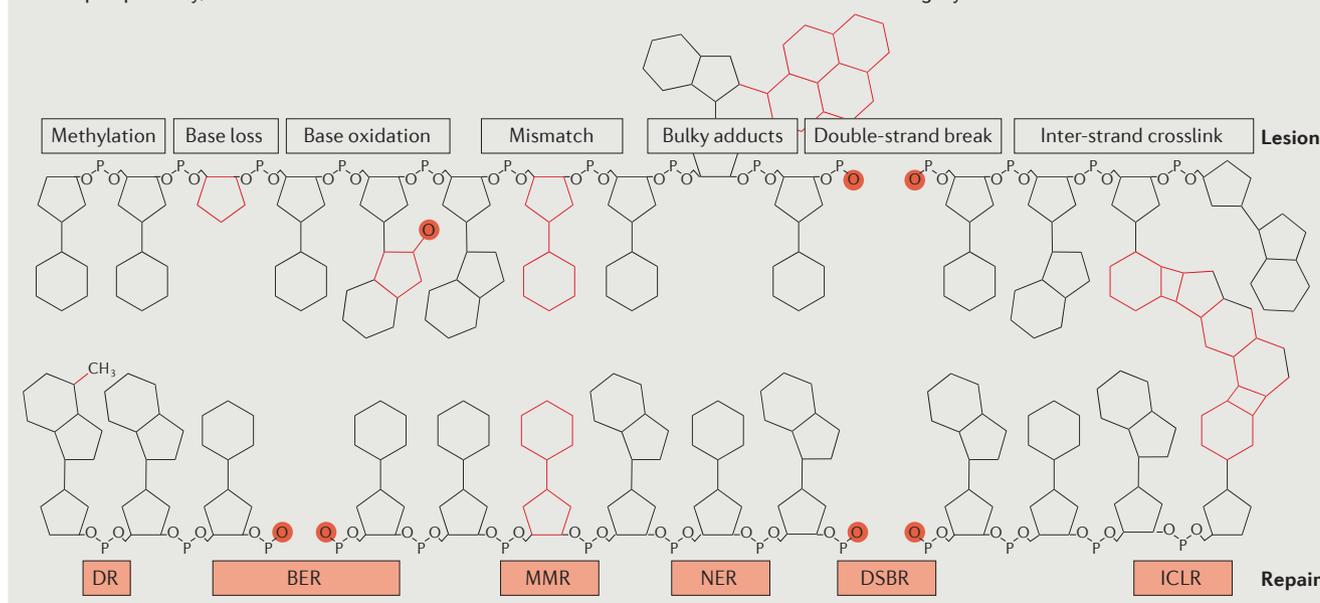


**Figure 1 | An overview of DNA damage-induced nucleus-to-mitochondria signalling and ageing.** Nuclear DNA damage can cause mitochondrial dysfunction. DNA damage leads to the activation of a number of proteins, resulting in widespread downstream changes in various cellular pathways. Among these pathways, signalling from the nucleus to mitochondria (NM signalling) is less studied than many others, but it may be very important in the ageing process and in age-associated diseases, and interventions in this process may slow ageing. A number of factors contribute to NM signalling, such as upstream DNA damage sensors including poly(ADP-ribose) polymerase 1 (PARP1), ataxia telangiectasia mutated (ATM) and the transcription factor p53. The NAD-dependent protein deacetylase sirtuin 1 (SIRT1) and the transcription regulator AMP-activated protein kinase (AMPK) then propagate the NM signal through post-translational modifications (deacetylation or phosphorylation, respectively) of DNA histones, peroxisome proliferator-activated receptor- $\gamma$  co-activator 1 $\alpha$  (PGC1 $\alpha$ ) and other proteins. The combined effect of the activation of the DNA damage response is downstream changes in the transcriptome, epigenome and metabolome, and in cellular bioenergetics. These contribute to ageing and the development of age-associated diseases such as neurodegeneration and cancer.

## Box 1 | Nuclear and mitochondrial DNA damage repair

Nuclear DNA damage is regarded as a major culprit in cancer, neurodegeneration, mitochondrial dysfunction and many age-related diseases, and unrepaired DNA damage can result in pathological levels of nucleus-to-mitochondria signalling (NM signalling), which may exacerbate disease progression. As DNA is constantly assaulted by both endogenous (for example, reactive oxygen species and hydrolysis) and exogenous (such as ultraviolet and ionizing radiation) stresses, cells have evolved several DNA repair pathways to maintain the integrity of DNA<sup>3,4,141–144</sup>. Major DNA repair pathways in the nucleus include direct reversal (DR) of the lesion (usually methylation), base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER; comprising global genome NER and transcription-coupled NER), double-strand break repair (DSBR, which includes non-homologous end-joining (NHEJ) and homologous recombination (HR)) and inter-strand crosslink repair (ICLR), among others (see the figure). DNA repair pathways in mitochondria are less well understood; the available data suggest that BER is the major DNA repair pathway, whereas NER does not exist in mitochondria.

DR, MMR and DSBR may exist in mitochondria, although their enzymatic constituents remain to be elucidated<sup>3,145,146</sup>. Intriguingly, a number of predominantly nuclear DNA repair proteins are also present in mitochondria. Many of the proteins involved in BER are present in both compartments, and for many other DNA metabolic proteins, it is unclear whether they are normally present in the mitochondria or imported under conditions of stress. Some DNA repair proteins, such as RECQL4, petite integration frequency 1 (PIF1), DNA replication helicase/nuclease 2 (DNA2) and Suppressor of Var1 3-like protein 1 (SUPV3L1) are localized at both the nucleus and mitochondria and may maintain mitochondrial DNA stability and/or mitochondrial function, in addition to promoting nuclear DNA integrity<sup>147,148</sup>. For example, RECQL4 is one of the five human RecQ helicases, and it is mutated in Rothmund–Thomson syndrome. RECQL4 primarily localizes to the nucleus and participates in nuclear DNA repair. A portion of RECQL4 also localizes to the mitochondria and regulates the translocation of p53 to mitochondria, as well as maintaining mitochondrial DNA integrity<sup>147,148</sup>.



clinical manifestations including severe early-onset neurodegeneration, hearing loss, muscle weakness and cachectic dwarfism<sup>44</sup>. This is caused by mutations in genes encoding Cockayne syndrome group A or B (CSA or CSB) proteins, which are also involved in the NER pathway of DNA repair. Recent studies in patient cells and various experiments in animal models suggest that both XPA and Cockayne syndrome have a noticeable phenotype of mitochondrial disease, as evidenced by severe mitochondrial clinical features and dysfunction including altered mitochondrial metabolism and accumulation of damaged mitochondria<sup>12,21,35</sup>. In patient cells and animal models of XPA and CSB, there is a reduction of SIRT1 activity, owing to persistent activation of PARP, induced by unrepaired DNA damage. Inhibition of PARP1 or supplementation of NAD<sup>+</sup> precursors restored SIRT1 activity and improved mitochondrial homeostasis<sup>12,21,35</sup>. Thus, short-term activation of PARP1 is beneficial for genomic stability, because it promotes DNA repair; however, persistent activation of PARP1 in age-related diseases probably contributes to pathology.

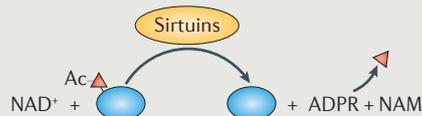
In addition to the NAD<sup>+</sup>–SIRT1–PGC1 $\alpha$  axis, other processes are affected by PARP1/SIRT1 competition. SIRT1 modulates the activities of mitochondria-related transcription factors other than PGC1 $\alpha$ , including HIF1 $\alpha$ , FOXO1 and FOXO3A, through deacetylation or degradation (for HIF1 $\alpha$ )<sup>45,46</sup>. DAF-16, a homologue of mammalian FOXO3A, is the only FOXO family member in *Caenorhabditis elegans*. The *C. elegans* SIR-2.1 (a homologue of mammalian SIRT1) can activate the DAF-16–superoxide dismutase (SOD-3) pathway to protect against reactive oxygen species (ROS)<sup>16</sup>, which can trigger PARP1 (FIG. 2). Many other pathway steps in NM signalling following DNA damage are being investigated.

**SIRT1 participates in nuclear DNA repair.** SIRT1 affects multiple DNA repair pathways. It deacetylates and activates some major DSBR proteins, including KU70, nibrin, histone deacetylase 1 (HDAC1) and the RecQ helicase protein Werner (WRN)<sup>47–49</sup>. In neurons, SIRT1 has a role in DSBR through non-homologous

Reactive oxygen species (ROS). By-products of cellular metabolism, which at low levels provide health benefits, whereas at high levels they become increasingly noxious, with broad pathological consequences.

Box 2 | Functions of sirtuins in ageing and age-associated diseases

Sirtuins are NAD<sup>+</sup>-dependent deacetylase and deacylase enzymes that control metabolism and ageing. Sirtuins modify proteins, and, in the process, they consume NAD<sup>+</sup> and generate nicotinamide (NAM) and ADP-ribose (ADPR; see the figure).



The *Saccharomyces cerevisiae* *SIR2* gene (silent information regulator 2; encoding the protein Sir2p) was the first sirtuin to be discovered; it affects replicative lifespan in yeast through histone deacetylation and chromatin silencing at ribosomal DNA repeats and sub-telomeric sequences<sup>33,149,150</sup>. The *SIR2* orthologue in *Caenorhabditis elegans* is *sir-2.1*. Overexpression of *sir-2.1* extended lifespan in some studies but not others<sup>16,151–153</sup>. There are seven mammalian sirtuins, SIRT1 to SIRT7. SIRT1, which is a nuclear protein, is defective in many age-associated diseases and regulates diverse ageing-related processes, including mitochondrial homeostasis and genomic stability<sup>12,33</sup>. More than 50 proteins are deacetylated by SIRT1, including some that are involved in energy metabolism (such as peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) and PPAR $\gamma$ ), mitochondrial homeostasis (peroxisome proliferator-activated receptor- $\gamma$  co-activator 1 $\alpha$  (PGC1 $\alpha$ ), hypoxia inducible factor 1 $\alpha$  (HIF1 $\alpha$ ), autophagy-related protein 7 (ATG7) and ATG8), and some that are involved in DNA repair (such as ataxia telangiectasia mutated (ATM), xeroderma pigmentosum group A-complementing protein (XPA), Werner syndrome ATP-dependent helicase (WRN), KU70 and others)<sup>12,33,47,48,154</sup>. SIRT2 is primarily cytoplasmic but is observed in the nucleus during mitosis, where it regulates the mitotic checkpoint. SIRT3, SIRT4 and SIRT5 are mitochondrial enzymes, with SIRT3 being responsible for the majority of deacetylation of mitochondrial metabolic enzymes<sup>155</sup>. The nuclear SIRT6 is linked to telomere maintenance and genome stabilization. In mice, SIRT6 loss leads to genomic instability and premature ageing phenotypes, whereas overexpression of SIRT6 extends mouse lifespan<sup>54,55,60,62</sup>. SIRT7, which is also a nuclear protein, promotes epigenetic stabilization of cancer-associated gene expression programmes and regulates mitochondrial homeostasis through the regulation of nuclear-encoded mitochondrial genes and the mitochondrial unfolded protein response (UPR<sup>mt</sup>)<sup>25,68</sup>. Mammalian sirtuins affect cancer biology through tumour suppressive and oncogenic effects<sup>33</sup>. Thus, the sirtuins participate in multiple cellular pathways, and future work should further interrogate the crosstalk between sirtuins and ageing.

end-joining (NHEJ). Specifically, it participates in the initial sensing and signalling of DSBs through stabilization of ataxia telangiectasia mutated (ATM) at DSB sites and deacetylates HDAC1, which is implicated in another step of NHEJ<sup>47</sup>. In proliferating cells, SIRT1 participates in homologous recombination and BER through deacetylation of WRN<sup>49</sup>. Even though ATM is a crucial sensor of DSBs, SIRT1 can promote DSB repair in an ATM-independent manner, which may involve WRN and other proteins<sup>50</sup>. SIRT1 also increases BER activity through deacetylation of key pathway proteins, including apurinic/apyrimidinic endonuclease 1 and thymine DNA glycosylase<sup>51,52</sup>. SIRT1 also promotes NER through deacetylation of XPA, resulting in enhanced interaction of XPA with other NER proteins<sup>53</sup>. Thus, in addition to direct regulation of mitochondrial homeostasis through the aforementioned pathways, SIRT1 promotes genomic stability (FIG. 2).

**Other sirtuins.** Two other nuclear sirtuins, SIRT6 and SIRT7, have important roles in chromatin regulation and genome maintenance and, like SIRT1, have intriguing links to mitochondrial homeostasis. SIRT6 is a chromatin-remodelling factor that maintains genomic stability through various mechanisms and represses gene expression programmes associated with ageing,

metabolism and cancer. SIRT6 is implicated in longevity regulation: overexpression of SIRT6 increases lifespan in male transgenic mice, and SIRT6 deficiency leads to shortened mouse lifespan, genomic instability and, in mouse cancer models, to increased tumour growth and aggressiveness<sup>54–56</sup>. Additionally, ageing-associated phenotypes observed in SIRT6-deficient mice have some features similar to those of Hutchinson–Gilford progeria syndrome (HGPS), which is a rare and fatal human premature ageing disease caused by mutations in the lamin A gene<sup>55,57,58</sup>. Lamin A may be an endogenous activator of SIRT6 by promoting its DNA repair-mediating activity<sup>58</sup>. Despite its nuclear localization and functions, SIRT6 influences mitochondrial homeostasis and has important effects on cancer cell metabolism. SIRT6 increases mitochondrial respiration by repressing transcription of key glycolytic enzymes through inhibition of HIF1 $\alpha$ ; in cancer cells, this prevents tumorigenic glycolytic metabolism (known as the ‘Warburg effect’)<sup>56,59</sup>.

SIRT6 also regulates genomic stability and promotes DSB repair through several mechanisms. It prevents telomere dysfunction and interacts physically or functionally with many DSB repair proteins, including PARP1, DNA-dependent protein kinase (DNA-PK), C-terminal-binding protein-interacting protein (CTIP), WRN and the ATP-dependent chromatin remodeller SWI/SNF-related matrix-associated actin-dependent regulator of chromatin A5 (SNF2H)<sup>60–64</sup>. Thus, SIRT6 is involved in DNA damage recognition<sup>64</sup> and affects mitochondrial homeostasis and tumour suppression. In addition to DNA repair, anti-ageing effects of SIRT6 may be associated with its role in repressing long interspersed element 1 (LINE1) retrotransposons, a group of transposable elements that induce genomic instability<sup>65</sup>. LINE1 transposition increases upon DNA damage or cellular and tissue ageing and is associated with depletion of SIRT6 at LINE1 promoters. Interestingly, retrotransposons can trigger innate immune responses, which may involve mitochondrial stress signalling<sup>1</sup>, thus suggesting linkage between SIRT6 and mitochondria. This area of research is a ripe topic for future investigations to better understand the role of SIRT6 in DNA damage-induced signalling.

SIRT7 is less well characterized than SIRT6, but studies indicate that it is a dynamic nuclear regulator of both chromatin and mitochondrial functions. SIRT7 promotes chromatin-dependent repression of genes involved in the epigenetic maintenance of oncogenic transformation, including cytoplasmic and mitochondrial ribosomal proteins<sup>66</sup>. In SIRT7-deficient mouse liver, increased ribosomal protein expression is associated with endoplasmic reticulum (ER) stress and hepatosteatosis<sup>67</sup>, and, in haematopoietic stem cells, SIRT7 loss leads to increased levels of mitochondrial ribosomal proteins and mitochondrial protein folding stress<sup>25</sup>. SIRT7 may regulate mitochondrial homeostasis by deacetylating GA-binding protein  $\beta$ 1 (GABP $\beta$ 1), which is a master regulator of nuclear-encoded mitochondrial genes; accordingly, SIRT7-deficient mice exhibit multi-systemic mitochondrial dysfunction<sup>68</sup>.

**Ataxia telangiectasia mutated (ATM).** A 350 kDa Ser/Thr protein kinase that is required for activation of the DNA damage response to double-strand breaks through phosphorylation of > 700 downstream DNA repair proteins.

**Hepatosteatosis**  
Also known as hepatic steatosis (fatty liver). A common liver abnormality, in which patients have excessive accumulation of triglycerides (lipid droplets) in the liver.

**Apurinic and apyrimidinic sites**

(AP sites; also known as abasic sites). Sites of DNA sugar without a base, which is a common DNA lesion and is typically repaired by DNA base excision repair through sugar cleavage by AP endonuclease 1 (APE1).

**8-oxo-dGuo**

(8-oxo-7,8-dihydro-2'-deoxyguanosine). An oxidative DNA lesion, which can be repaired by DNA base excision repair.

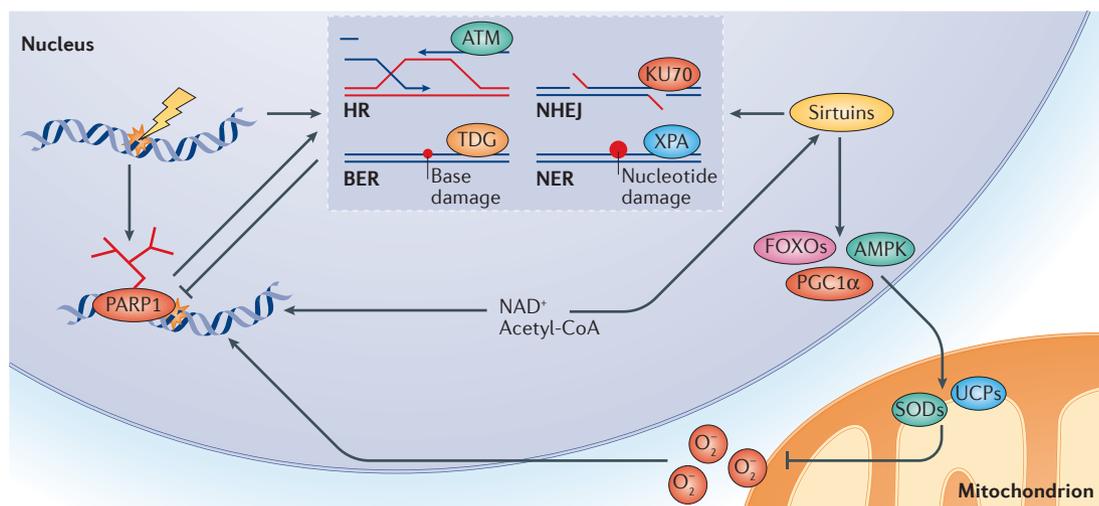
In addition to nuclear sirtuins, the mitochondrial SIRT3 and SIRT4 may participate in DNA damage-induced NM signalling. SIRT3, a major mitochondrial deacetylase, prevents both age-related and noise-induced hearing loss through reduction of oxidative mtDNA damage (such as apurinic and apyrimidinic sites and 8-oxo-dGuo) in the cochlea and brain, or through reduction of neurite degeneration caused by noise<sup>69,70</sup>. The roles of SIRT3 in neuroprotection may be mediated by augmentation of the mitochondrial antioxidant defence system and deacetylation of several proteins, including mitochondrial isocitrate dehydrogenase 2, SOD2 and cyclophilin D<sup>69,71</sup>. SIRT4 is induced by many genotoxic agents, and it regulates the cellular metabolic response to nuclear DNA damage response pathways through repression of mitochondrial glutamine metabolism. Although the roles of mitochondrial sirtuins in ageing, metabolism and mitochondrial function have been established<sup>33,72,73</sup>, further work on how these mitochondrial sirtuins link mitochondrial maintenance and genomic stability will improve our understanding of their roles in NM signalling.

Although we discuss a few of the nuclear sirtuins separately, there is evidence for functional interactions among them. The expression of SIRT6 may be dependent on SIRT1 through the formation of a SIRT1–FOXO3A–nuclear respiratory factor 1 (NRF1) transcription complex on the SIRT6 promoter<sup>74</sup>. SIRT7

and SIRT1 interact physically, and this interaction is important for regulation of the epithelial–mesenchymal transition (EMT)-like process of metastatic progression in cancer cells<sup>75</sup>. Proteomic studies of SIRT6- or SIRT7-interacting factors uncovered more than 110 common proteins, although it is not known whether these interactions occur in the same or in distinct protein complexes<sup>76</sup>. Several of these proteins, including nucleophosmin and nucleolin, are associated with ageing-related processes such as DNA repair and cellular senescence, but they can also have an impact on mitochondrial biology<sup>76</sup>, indicating a possible involvement in DNA damage-induced NM signalling.

**Metabolic NM signalling pathways**

DNA damage leads to metabolic alterations at the cellular and organismal levels. This is evident in premature ageing disorders such as Werner syndrome, ataxia telangiectasia, Cockayne syndrome and Hutchinson–Gilford progeria, in which weight loss is highly prevalent<sup>35,77,78</sup>. Notably, weight loss is common in normal human ageing, as well as in neurodegenerative diseases such as Parkinson disease, Huntington disease and amyotrophic lateral sclerosis (ALS)<sup>79</sup>. Indeed, in cell and animal models of accelerated ageing, weight loss is prominent and may be caused by increased metabolic rates<sup>12,35,80</sup>. Pharmacological inhibition of the DNA damage sensor PARP1 decreases the metabolic rate of old *Csb<sup>tm/m</sup>* mice



**Figure 2 | PARP1–NAD<sup>+</sup>–SIRT1-mediated nuclear DNA damage to mitochondrial signalling.** Activation of poly(ADP-ribose) polymerase 1 (PARP1) upon DNA damage facilitates DNA repair but leads to loss of NAD<sup>+</sup> and acetyl-CoA, the latter being an important molecule in cellular metabolism. This results in inhibition of sirtuins such as SIRT1, owing to competition for NAD<sup>+</sup>. Loss of sirtuin activity leads to an increase in mitochondrial reactive oxygen species (O<sub>2</sub><sup>•-</sup>), owing to decreased activation of downstream stress response factors such as AMP-activated protein kinase (AMPK), forkhead box O proteins (FOXOs) and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α). Collectively, these enzymes regulate a large number of genes that are involved in coping with oxidative stress, such as those encoding uncoupling proteins (UCPs) like UCP2 and superoxide dismutases (SODs). In addition, SIRT1, as well as SIRT6, has been shown to positively regulate several DNA repair pathways, such as homologous recombination (HR), non-homologous end-joining (NHEJ), base excision repair (BER) and nucleotide excision repair (NER). Specifically, SIRT1 has been shown to deacetylate repair factors such as xeroderma pigmentosum group A-complementing protein (XPA, which is involved in NER), KU70 (involved in NHEJ), ataxia telangiectasia mutated (ATM; involved in HR and NHEJ) and thymine DNA glycosylase (TDG; involved in BER). Activation of PARP1 and subsequently less-robust DNA repair, as well as increased oxidative stress, may represent a vicious cycle that could contribute to the ageing process. Thus, PARP1 is pivotal to the initiation of different DNA repair pathways, and it interacts with SIRT1 to execute cellular signalling from the nucleus to mitochondria.



### Mitophagy–apoptosis crosstalk

DNA damage activates certain pivotal proteins that lead to amplification and propagation of a signal, ultimately resulting in cellular outcomes such as mitophagy and apoptosis. Several mediators seem to be important for the choice between autophagy and mitophagy or apoptosis. Importantly, low levels of DNA damage stress may stimulate mitophagy and antagonize apoptosis, whereas high levels of stress inhibit mitophagy and stimulate apoptosis.

Mitochondria not only have a central role in metabolic pathways, they also regulate cell fate through crosstalk between mitophagy and apoptosis. Macroautophagy (autophagy) and apoptosis are intimately interconnected to determine whether cells survive or die (FIG. 4). DNA damage constitutes a strong intrinsic apoptotic signal. Apoptosis can occur by several different mechanisms, including caspase 8-dependent apoptosis, mitochondria-mediated caspase 9- and caspase 3-dependent apoptosis, and caspase-independent necrosis or autophagic cell death<sup>90</sup> (FIG. 4a). The B cell lymphoma 2 (BCL-2) family of proteins are closely involved in the regulation of apoptosis and consist of both pro-survival members, including BCL-2, BCL-XL, BCL-W, A1A and MCL2; and pro-apoptotic members such as BAX, BAK, BIM, BID (BCL-2 homologous 3-interacting domain death agonist) and PUMA<sup>91</sup>. Apoptosis is initiated when the DNA lesion burden exceeds a specific threshold, leading to the initiation of intrinsic and/or extrinsic apoptotic pathways.

Autophagy (*auto*, self; *phagy*, eating of) generally functions as a pro-survival mechanism, and defective autophagy is linked with neurodegenerative diseases and ageing. Autophagy is a process whereby cells sequester damaged or unused cytoplasmic substrates within a double-membrane vesicle — the autophagosome — that is then fused with lysosomes, leading to the degradation of the engulfed substrates<sup>92</sup>. Mitophagy is the selective degradation of mitochondria by autophagy. In mammalian cells, there are several mitophagy pathways, such as NIX (also known as BNIP3L)-regulated programmed mitophagy, which is found in blood cells; and selective mitophagy, which can be regulated in a PTEN-induced putative kinase 1 (PINK1)-dependent or PINK1-independent manner<sup>3,93–95</sup>. Genes encoding PINK1 and the ubiquitin ligase parkin are often mutated in autosomal recessive parkinsonism<sup>5</sup>. In mitophagy, full-length PINK1 located at the outer mitochondrial membrane phosphorylates ubiquitin to activate parkin, which generates ubiquitin chains to recruit downstream autophagy receptors and signals robust mitophagy<sup>95</sup> (FIG. 4b). In normal, steady-state conditions, the mitochondrial membrane potential drives full-length PINK1 through the translocate of the inner membrane (TIM) complex, where mitochondrial processing peptidase (MPP) and presenilin-associated rhomboid-like protein (PARL) cleave the mitochondrial targeting sequence and transmembrane domain of PINK1. The cytoplasmic part of PINK1 is further degraded by the ubiquitin proteasome<sup>5,12,96</sup>. Thus, mitophagy targets damaged and depolarized mitochondria, as mitochondria with higher mitochondrial membrane potentials are spared from PINK1-dependent mitophagy.

It is increasingly clear that mitophagy plays an important part in the maintenance of neuronal health, as well as in the ageing process itself<sup>11</sup>. Current knowledge of the relationship between autophagy and apoptosis suggests that autophagy precedes apoptosis in many instances, and that autophagy and apoptosis are often antagonistic<sup>22,90,92</sup> (FIG. 4). In this section, the interplay between classic apoptotic mediators and mitophagy, and the role of NM signalling in the regulation of this crosstalk, will be illustrated in three major pathways.

**ATM-mediated mitophagy–apoptosis crosstalk.** The protein kinase ATM is a major activator of the DNA damage response. It has a key role in the repair of DSBs, and it is closely linked to health through its roles in the maintenance of genomic stability and mitochondrial homeostasis (FIG. 4c). ATM triggers multiple cellular events to promote cell survival at low levels of DSBs; conversely, it drives cellular apoptosis after extensive DSB damage<sup>23,97</sup>. ATM promotes survival by activating repair of DSBs, upregulating pro-survival genes and inducing autophagy and mitophagy. In response to DNA damage, cells recruit DNA repair proteins, including ATM, to DSBs to initiate DNA repair. This process also involves ubiquitylation of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) essential modifier (NEMO) protein, which translocates from the nucleus to the cytoplasm, where it activates the Jun N-terminal kinase (JNK)<sup>97,98</sup>. JNK modulates the balance between apoptosis and autophagy and mitophagy through the phosphorylation of specific BCL-2 protein family members, such as BCL-2 and BIM. This releases the pro-autophagy protein Beclin 1 from BCL-2 or BIM complexes, and autophagy is initiated<sup>99</sup>. When DSBs are extensive, JNK instead promotes apoptosis through at least two independent pathways: the inactivation of the anti-apoptotic BCL-2 and the activation of the FAS-associated death domain protein (FADD)–caspase 8 pro-apoptotic pathway<sup>90,97</sup>. Thus, in response to DSBs, ATM uses NEMO and JNK to regulate autophagy/mitophagy–apoptosis crosstalk (FIG. 4c). The ATM–BID axis is an additional regulator of mitochondrial function following DNA damage. ATM may inhibit ROS production by phosphorylating BID and preventing its translocation to mitochondria and apoptosis<sup>100</sup> (FIG. 4c).

A role for ATM in mitochondrial homeostasis and longevity is supported by the phenotypes of ataxia telangiectasia patients and *Atm*<sup>−/−</sup> laboratory animal models. Patients with ataxia telangiectasia show neurodegeneration, cancer predisposition and sterility<sup>23</sup>. Cells from these patients have mitochondrial dysfunction and alterations in mtDNA copy number, higher mitochondrial levels of ROS, elevated cellular respiratory capacity and an accumulation of damaged mitochondria<sup>12,80,101</sup>. The importance of mitochondrial dysfunction in the aetiology or progression of ataxia telangiectasia is further supported by the notion that ataxia telangiectasia patient cells and *Atm*<sup>−/−</sup> mouse models are highly sensitive to oxidants. Conversely, mitochondrial antioxidants protect *Atm*<sup>−/−</sup> cells against oxidative stress and mitigate some ataxia telangiectasia phenotypes in *Atm*<sup>−/−</sup> mice<sup>23</sup>. It is likely that defective

#### Ketogenic diet

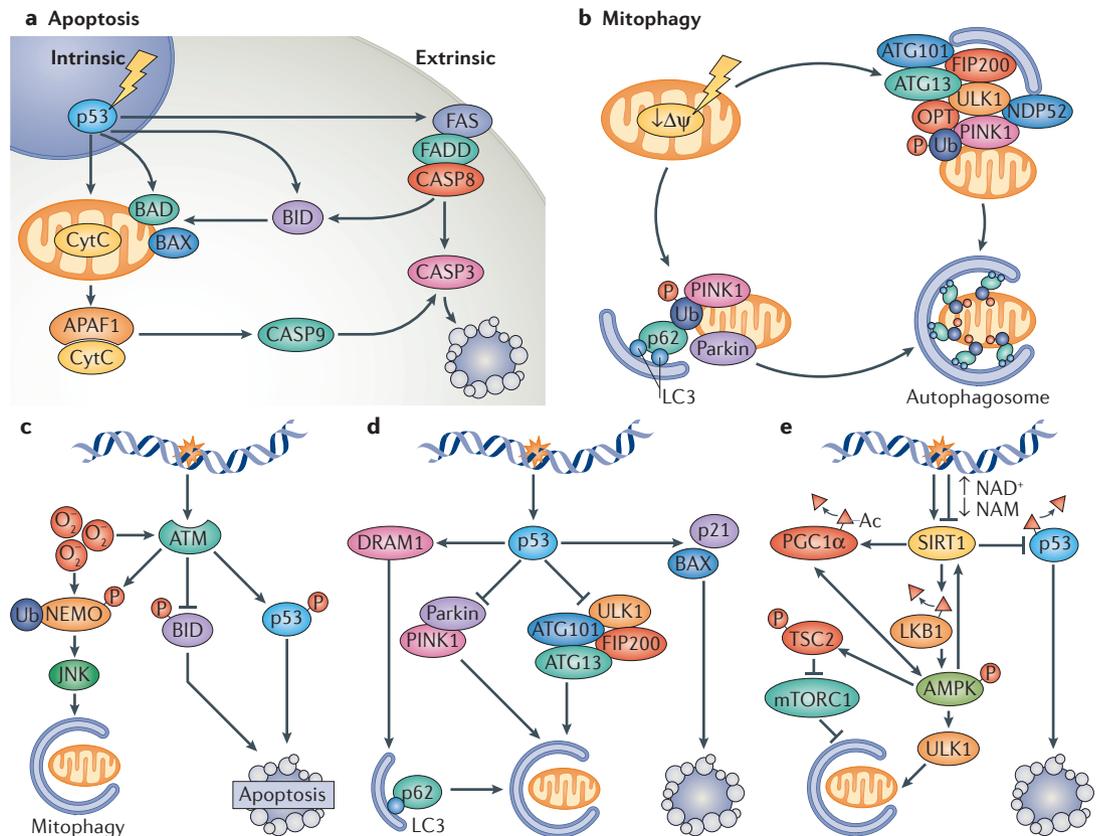
A diet that is high-fat, high-protein and low-carbohydrate.

#### Stress response

A cellular response to certain types of stress, such as caloric restriction or increase in reactive oxygen species, in which different stress-counteracting pathways are upregulated.

#### Autophagosome

A key structure of autophagy, an autophagosome is a spherical, double-membrane vesicle that sequesters cytoplasmic contents for degradation.



**Figure 4 | DNA damage signalling in the regulation of mitophagy and apoptosis.** **a** | Apoptosis can be initiated through intrinsic or extrinsic pathways. The intrinsic pathways can be initiated by genotoxic stress, leading to the activation of p53 and transcriptional upregulation of pro-apoptotic factors such as BAD, BAX and BID. If the stress is sustained or high enough, BAD and BAX accumulation results in the permeabilization of the outer mitochondrial membrane and release of cytochrome C (CytC) that can associate with apoptosis-activating factor 1 (APAF1) and activate caspase 9 (CASP9). CASP9 activation leads to CASP3 activation and initiation of apoptosis. The extrinsic pathway can be initiated through activation of the FAS receptor. This leads to FAS-associated death domain protein (FADD)-mediated activation of CASP8, resulting in CASP3 activation. Notably, there is considerable crosstalk between intrinsic and extrinsic pathways. **b** | Selective mitophagy is regulated through factors such as PTEN-induced putative kinase 1 (PINK1) and the E3-ubiquitin ligase parkin. Initially, mitochondrial damage causes loss of the mitochondrial membrane potential ( $\Delta\psi$ ). This leads to the retention of PINK1 at the outer mitochondrial membrane and phosphorylation of outer membrane proteins. Concomitantly, parkin is activated, leading to ubiquitylation of outer membrane proteins. PINK1 phosphorylates ubiquitin that recruits adaptor proteins such as optineurin (OPT), nuclear dot protein 52 (NDP52) and p62, leading to recruitment of the UNC51-like kinase 1 (ULK1) complex (consisting of proteins such as autophagy-related protein 101 (ATG101), ATG13 and FIP200) and to the formation of a double lipid membrane-bound vesicle, the autophagosome. The adaptor proteins (OPT, p62 and NDP52) interact with the autophagosome through light chain 3 (LC3), a small protein that coats the autophagosome. The entire mitochondrion will eventually become engulfed in the autophagosome, which will fuse with a lysosome leading to degradation of the mitochondrion. **c** | Ataxia telangiectasia mutated (ATM) is activated by breaks in DNA and possibly also by oxidative stress ( $O_2^-$ ). At low levels of DNA damage stress, ATM activation leads to phosphorylation, ubiquitylation and activation of the NEMO–JNK (NF- $\kappa$ B essential modulator–Jun N-terminal kinase) pathway that stimulates mitophagy. ATM also phosphorylates the pro-apoptotic factor BID to inhibit apoptotic signalling. At high levels of stress, ATM phosphorylates and activates p53, which propagates pro-apoptotic signals. **d** | p53 has been well characterized in the response to genotoxic stimuli, in which it transcriptionally activates pro-apoptotic proteins such as BAX and p21 while simultaneously inhibiting the ULK1-containing autophagy-initiating complex. In addition, p53 activation may lead to decreased expression of parkin and decreased activation of parkin–PINK1-mediated mitophagy. At lower levels of stress, p53 can stimulate mitophagy through the activation of DNA damage-regulated autophagy modulator protein 1 (DRAM1), which stimulates p62-mediated mitophagy. **e** | Sirtuin 1 (SIRT1) regulates both mitophagy and apoptosis. At low levels of DNA damage, nuclear SIRT1 can be activated to facilitate DNA repair. After lethal levels of nuclear DNA damage, SIRT1 is inhibited by the DNA damage response, leading to p53 acetylation and cell death. Loss of SIRT1 activity decreases the stimulation of mitophagy through peroxisome proliferator-activated receptor- $\gamma$  co-activator 1 $\alpha$  (PGC1 $\alpha$ )- and AMP-activated kinase (AMPK)-dependent pathways. SIRT1 regulates mitochondrial biogenesis and mitophagy through deacetylation of PGC1 $\alpha$ , and it also interacts with AMPK to regulate mitophagy through mutual activation: AMPK activates SIRT1 by increasing the ratio of  $NAD^+$ /NADH, and it is activated by SIRT1 through deacetylation of liver kinase B1 (LKB1), an AMPK activator. Furthermore, AMPK phosphorylates and activates the mTORC1 inhibitor protein tuberous sclerosis complex 2 (TSC2), thereby further activating autophagy. AMPK also positively regulates PGC1 $\alpha$  activity, which stimulates mitochondrial biogenesis and activates forkhead box protein O 3A, leading to activation of stress response and pro-survival pathways. NAM, nicotinamide.

mitophagy significantly contributes to the mitochondrial dysfunction in ataxia telangiectasia<sup>12,80</sup>. Using a bioinformatics tool, *Mito DB*, it was reported that patients with ATM mutations clinically resemble patients with mitochondrial diseases<sup>12,102</sup>. Further studies into pathways involved in mitochondrial dysfunction in ataxia telangiectasia are warranted.

***p53-mediated mitophagy–apoptosis crosstalk.*** The tumour suppressor p53 is at the hub of numerous signalling pathways, but although the role of p53 in apoptosis is well established, it has only recently been associated with mitophagy (FIG. 4d). Under lethal genotoxic stress, p53 transcriptionally upregulates pro-apoptotic proteins such as p21, RB, BAX, FAS, death receptor 5 (DR5; also known as TNFRSF10B), NOXA, PIG3, p53AIP1 and PUMA, which leads to cellular apoptosis, senescence or growth arrest<sup>103,104</sup>. Of note, p53 in the cytoplasm may enhance apoptosis through inhibition and destabilization of the autophagy-initiation ULK1–FIP200–autophagy-related protein 13 (ATG13)–ATG101 complex, as well as through inhibition of PINK1–Parkin-mediated mitophagy<sup>105</sup>. However, in cells under low levels of stress, nuclear p53 can bind the promoter regions of genes that encode pro-autophagy proteins, including DNA damage-regulated autophagy modulator 1 (DRAM1), thereby inducing mitophagy<sup>89,106</sup>. DRAM1 is a lysosomal membrane protein involved in autophagy induction; when mitochondrial protein synthesis is impaired, mitochondria generate more ROS to activate autophagy and mitophagy through the ROS–p53–DRAM1 pathway<sup>107</sup>. These examples illustrate how p53-mediated nuclear signalling regulates apoptosis and mitophagy (FIG. 4d).

In recent years, evidence has accumulated to suggest that p53 has crucial roles in ageing through interconnected regulation of apoptosis, senescence and autophagy. Studies from animal models support the view that p53 signalling prolongs lifespan, as evidenced by decreased levels of tumour incidence, senescence and ageing-associated damage in model organisms<sup>108–110</sup>. In *C. elegans*, it was reported that *daf-2* (encoding an insulin-like receptor) mutants had extended lifespan through inhibition of tumour growth in a DAF-16–p53-dependent manner<sup>110</sup>. Compared with wild type, mice with moderately high levels of p53 showed high cancer resistance, increased antioxidant activity and delayed ageing<sup>109</sup>. In view of the role of p53 in the induction of autophagy, it would be of interest to investigate the role of autophagy in p53-mediated longevity. Collectively, the available data suggest that p53 can determine cell fate by influencing mitophagy–apoptosis crosstalk, and that an imbalance of this crosstalk in favour of apoptosis may contribute to degenerative diseases and ageing.

***SIRT1-mediated mitophagy–apoptosis crosstalk.*** In addition to the well-described role of SIRT1 in mitochondrial biogenesis through PGC1 $\alpha$ , SIRT1 also regulates the crosstalk between mitophagy and cell death. SIRT1 deacetylates and inactivates p53, allowing cells to escape apoptosis after excessive toxic stress, including DNA damage<sup>111,112</sup> (FIG. 4e). It also inhibits apoptosis

by activating autophagy and mitophagy. In yeast, Sir2 (silent information regulator 2; the homologue of mammalian SIRT1) protects against ageing, partially through induction of mitophagy by transcriptional upregulation of the mitophagy executor Atg32 (REF. 113). In mammals, SIRT1 can activate autophagy through several pathways, including deacetylation of light chain 3 (LC3; also known as MAP1LC3B), which facilitates its nuclear export and its conjugation on the autophagic membrane through interaction with another cytoplasmic autophagy regulator, ATG7 (REF. 114). SIRT1 can also regulate mitophagy by maintaining PINK1 integrity through regulation of the mitochondrial membrane potential<sup>12</sup>. In XPA, a high mitochondrial membrane potential destabilizes full-length PINK1, causing defective mitophagy. Activation of the NAD<sup>+</sup>–SIRT1–PGC1 $\alpha$  axis in XPA cells restored mitophagy through upregulation of mitochondrial uncoupling protein 2 (UCP2)<sup>12</sup>.

SIRT1 also interacts cooperatively with AMPK, which is a central regulator of metabolism and energy homeostasis, to stimulate mitophagy (FIG. 4e). Either physiological (through fasting or exercise) or pharmacological activation of AMPK can activate SIRT1 through increasing the NAD<sup>+</sup>/NADH ratio<sup>12,45,89</sup>. Interestingly, AMPK can also be activated by SIRT1 through deacetylation of LKB1, leading to the phosphorylation and activation of AMPK. This may suggest the existence of a positive-feedback loop in the regulation of SIRT1–AMPK signalling<sup>115</sup>. Thus, SIRT1 may counteract ageing by maintaining mitochondrial integrity and inhibiting apoptosis through tight regulation of several major cell fate-determining proteins. Importantly, hyperactivation of the DNA damage response may inhibit SIRT1 activity through the consumption of NAD<sup>+</sup>, even though SIRT1 promotes genomic stability through participation in DNA repair, as discussed above.

Collectively, autophagy and mitophagy are key regulators of mitochondrial homeostasis, and defective mitophagy may accelerate the ageing process and the development of age-related neurodegenerative diseases. Enhancement of autophagy and mitophagy may therefore represent a therapeutic strategy for these diseases.

### Strategies for improving healthspan

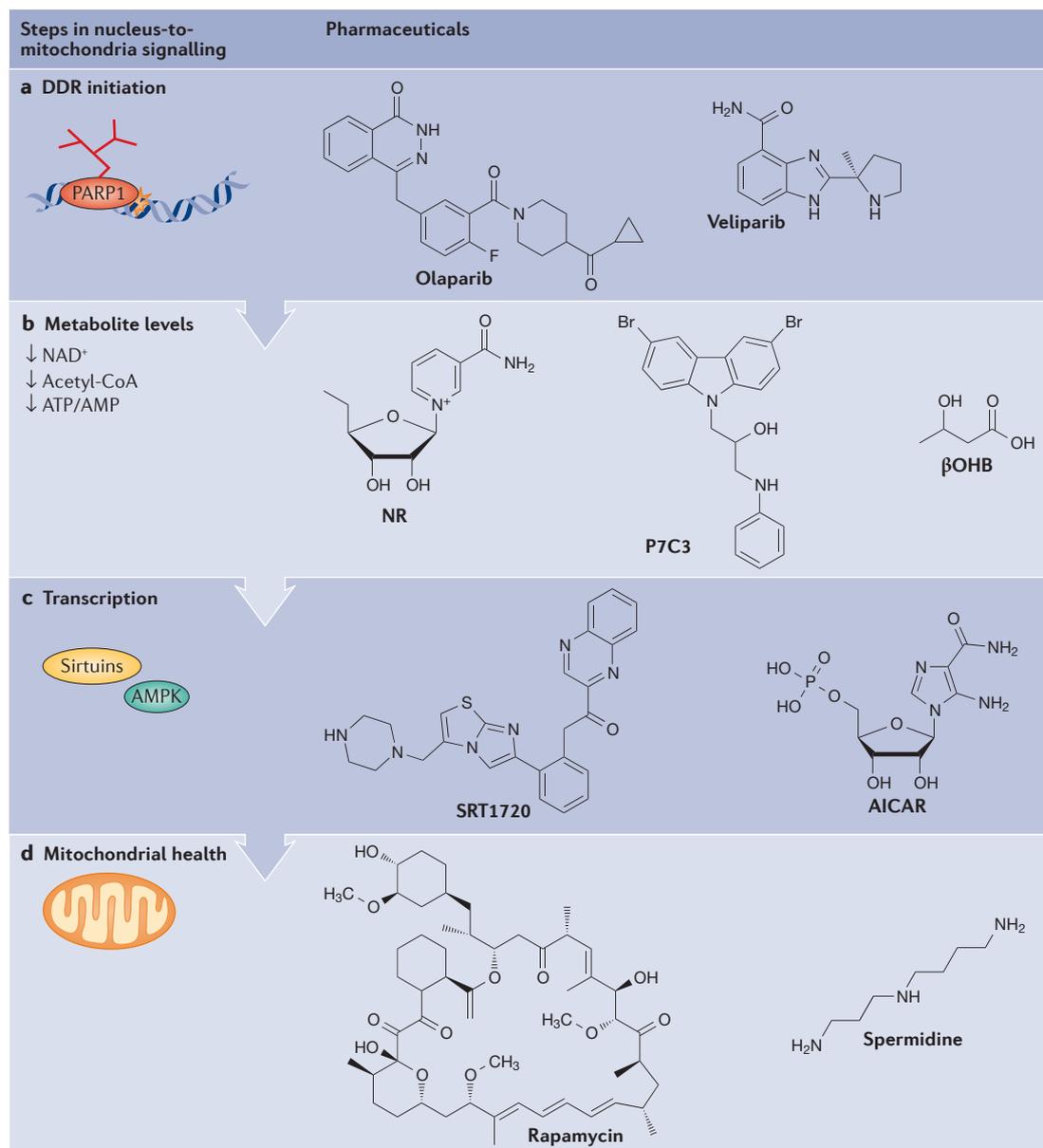
Recent findings demonstrate the possibility of interventions in ageing that will reduce or delay morbidity and increase healthspan<sup>116</sup>. The NM signalling network is emerging as a promising target for improving healthspan.

***Enhancing the NAD<sup>+</sup>–SIRT1 pathway.*** The NAD<sup>+</sup>–SIRT1 pathway is an important target for intervention. SIRT1 activity can be enhanced by increasing NAD<sup>+</sup> levels or by the use of SIRT1 activators. One way to increase NAD<sup>+</sup> levels is to reduce NAD<sup>+</sup> consumption by, for example, using inhibitors of PARPs (such as olaparib and veliparib (FIG. 5a)) or of other NAD<sup>+</sup>-consuming enzymes such as CD38 (REFS 12, 16). As PARP1 is a key protein in DNA repair, and because its inhibition may lead to genomic instability, it is necessary to proceed cautiously with such long-term intervention in humans. As CD38 participates in several cellular functions (such as immunity,

signal transduction and calcium signalling), and even though inhibition of CD38 may increase SIRT1 activity, the side-effects of CD38 inhibition are unclear and thus warrant caution when applying this strategy in humans<sup>27</sup>.

NAD<sup>+</sup> precursors such as nicotinamide riboside and nicotinamide mononucleotide (NMN) also increase SIRT1 activity and have potential as anti-ageing drugs (FIG. 5b). In *C. elegans*, NAD<sup>+</sup> levels are decreased during ageing, in part, by age-associated induction of NAD<sup>+</sup>-consuming PARP1. Consistently, supplementation with

nicotinamide riboside increased worm lifespan in a SIR2.1-dependent manner<sup>12,16</sup>. In 22-month-old mice, 7-day supplementation with NMN significantly rescued mitochondrial dysfunction and normalized the mitochondrial parameters to those of young mice<sup>46</sup>. Nicotinamide riboside was also reported to be beneficial in both *C. elegans* and mouse models of XPA and Cockayne syndrome, which exhibit impaired NM signalling<sup>12,35</sup>. Encouraged by these data, several nicotinamide riboside clinical trials are underway<sup>117–120</sup>. Intriguingly, the P7C3 class of



**Figure 5 | Pharmacological interventions in the DNA damage response that may lead to increases in lifespan and healthspan. a** | Inhibition of poly(ADP-ribose) polymerase 1 (PARP1) by olaparib and other PARP inhibitors has been shown to increase lifespan in model organisms. **b** | Impaired cellular metabolism can be counteracted by treatment with NAD<sup>+</sup> precursors such as nicotinamide riboside (NR), by activation of NAD<sup>+</sup>-generating enzymes such as nicotinamide phosphoribosyltransferase by P7C3, or by replenishment of acetyl-CoA levels with ketones such as  $\beta$ -hydroxybutyrate ( $\beta$ OHB). **c** | Signalling factors and targets in the DNA damage response (DDR) may constitute additional targets for interventions. These include activation of sirtuin 1 by compounds such as SRT1720, or AMP-activated kinase (AMPK) activation using the AMP analogue AICAR. **d** | Mitochondrial function may be augmented by stimulation of autophagy by rapamycin or its newer analogues, or by stimulation of autophagy through alternative pathways using compounds such as spermidine.

aminopropyl carbazole chemicals, which increase cellular NAD<sup>+</sup> levels by enhancing the activity of the NAD<sup>+</sup>-generating enzyme nicotinamide phosphoribosyltransferase (NAMPT), show strong neuroprotective activity<sup>121</sup>. Furthermore, NAMPT levels and SIRT1 activity are increased by treatment with AMPK activators such as 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribose (AICAR)<sup>45</sup> (FIG. 5c).

A group of small molecules called SIRT1-activating compounds (STACs) may also have some health benefits. STACs are either natural or synthetic small bioactive compounds, which allosterically activate SIRT1 by binding to the SIRT1-activation domain (amino acids 190–240)<sup>122,123</sup>. Some STACs, such as the natural polyphenol resveratrol and its synthetic analogues SRT2104 and SRT1720 (FIG. 5c), can extend healthspan and lifespan in mice, and some human studies have shown protective effects of resveratrol and SRT2104 (REFS 123–125). Ongoing laboratory and clinical studies to determine the long-term safety and clinical applicability of STACs are necessary before their potential as anti-ageing agents can be explored. Collectively, the development of NAD<sup>+</sup>-SIRT1 pathway pharmaceuticals is highly desirable.

**Ketones.** Ketones (or ketone bodies) are a group of molecules produced through fatty acid oxidation in the liver by the conjugation of two acetyl-CoA molecules. Importantly, ketogenesis is induced by fasting, which is a well-characterized pro-longevity intervention. Ketones enter the blood circulation and are transported into the brain and other organs, where they are used as substrates for energy production. Notably, ketones can rescue features of accelerated ageing in Cockayne syndrome and can attenuate neurodegeneration in models of ALS, Parkinson disease, Huntington disease and Alzheimer disease<sup>86,126</sup>. Indeed, a recent synthetic ketone ester was developed that ameliorated behavioural deficits and amyloid pathology in a mouse model of Alzheimer disease, thereby providing a new pharmacological approach for tackling age-associated neurodegenerative diseases<sup>126</sup>. Furthermore, the ketone body D- $\beta$ -hydroxybutyrate ( $\beta$ -OHB) (FIG. 5b) is a histone deacetylase inhibitor that was shown to extend lifespan in *C. elegans*, possibly through activating the SIR-2.1, AMPK, DAF-16–FOXO and SKN-1–NRF pathways<sup>127</sup>. The potential health benefits of  $\beta$ -OHB may be at least partially attributed to its ability to suppress oxidative stress by mediating increased histone acetylation at the *FOXO3A* and metallothionein 2 (*MT2*) promoters<sup>128</sup>. Although still speculative, bioenergetic interventions could thus be a way to decrease age-associated genome instability.

**Inducing mitophagy.** Inducing autophagy and mitophagy to diminish the load of damaged proteins and mitochondria, and to increase cell survival, may ameliorate age-related diseases accompanied by mitochondrial dysfunction. Common features of Alzheimer disease, Parkinson disease and Huntington disease include mitochondrial dysfunction, impaired autophagy and neuronal death<sup>13,92,129</sup>. Accumulation of damaged mitochondria may induce neuronal death in Parkinson

disease, and stimulation of autophagy can ameliorate disease pathology in laboratory Parkinson disease models<sup>13,92</sup>. In addition, defective mitophagy has been suggested as a trigger of disease progression, especially neurodegeneration, in some premature ageing diseases including XPA, Cockayne syndrome and ataxia telangiectasia<sup>12,35,80</sup>. In *C. elegans* and mouse models of XPA and Cockayne syndrome, increased mitophagy through restoration of the NAD<sup>+</sup>-SIRT1 (SIR2.1) pathway improves healthspan<sup>12,35</sup>.

Another major strategy to enhance autophagy and mitophagy is to inhibit mTORC1, which is a negative regulator of autophagy (FIG. 5d), using compounds such as rapamycin and associated rapamycin esters. Indeed, rapamycin can increase mouse lifespan and rejuvenate the ageing heart, and it is beneficial when administered in mouse models of Alzheimer disease, Parkinson disease, Huntington disease and Hutchinson–Gilford progeria; thus, it is being considered as an anti-ageing drug<sup>92,130,131</sup>. However, potential toxicity of rapamycin and its analogues have been noticed<sup>132</sup>, raising the need for discovery of new mTORC1-inhibiting compounds with fewer side-effects. New autophagy-inducing compounds that may function independently of mTORC1 have been identified, including spermidine (FIG. 5d) and some US Food and Drug Administration-approved drugs such as L-type Ca<sup>2+</sup>-channel antagonists and imidazoline receptor agonists<sup>133,134</sup>. Clearly, the next generation of autophagy stimulators could be promising drugs for various age-associated diseases.

### Open questions and future challenges

Impairment of DNA damage-mediated NM signalling can cause mitochondrial dysfunction, which is linked to ageing and various age-associated pathologies. Our understanding of the mechanisms of mitochondrial maintenance has greatly expanded over the decades, implicating both mitochondrial processes and signalling between mitochondria and other cellular compartments, especially the nucleus. Because of their central physiological role, mitochondria continue to be a focus of investigations in a great number of diseases and ageing.

In light of the importance of DNA damage-induced NM signalling in mitochondrial maintenance, genomic stability and longevity, associated pathways are of interest. For instance, modulation of DNA damage-induced NM signalling can activate UPR<sup>mt</sup>, which is a signalling pathway that contributes to longevity<sup>16,24–27</sup>. Inhibition of the DNA repair signalling protein PARP1 seems to extend lifespan in *C. elegans* through activation of UPR<sup>mt</sup> (REF. 16). There may be inducers of UPR<sup>mt</sup> other than DNA damage, and other ways to coordinate NM signalling with signalling from mitochondria to the nucleus. A related issue is the linkage between telomere maintenance and NM signalling. Telomere attrition may induce mitochondrial dysfunction through the p53–PGC1 $\alpha$  axis<sup>135</sup>. Some telomeric proteins, including telomerase reverse transcriptase and TRF1-interacting nuclear protein 2 (TIN2) may localize to both the nucleus and mitochondria and thereby link nuclear and mitochondrial events through DNA damage-induced NM signalling<sup>136,137</sup>.

#### Rapamycin

A natural metabolite from the bacterium *Streptomyces hygroscopicus*, which inhibits mTOR and extends lifespan in species from yeast to fruit flies and mice.

Additional mechanistic investigations are needed to propose a unified role for PARP1–sirtuin cross-talk in ageing. It seems contradictory that inhibition of PARP1 extends lifespan in both wild-type and disease animal models<sup>12,16</sup>, but that high PARP1 activity may be associated with longevity<sup>138,139</sup>. We hope that future studies will uncouple the conceptual link between PARP1-induced PARylation and high levels of DNA repair activity. High levels of PARP1-induced PARylation will facilitate DNA repair when the latter is proficient, but it will over-consume NAD<sup>+</sup> without enhancing lesion repair when DNA repair pathways are compromised. PARylation is regulated in a dynamic manner and can also be reversed by poly(ADP-ribose) glycohydrolase<sup>138</sup>. Thus, PARylation levels do not necessarily correlate with PARP activity. However, the sirtuin family of NAD<sup>+</sup>-dependent deacetylase and deacylase enzymes affect both intra-mitochondrial and NM signalling mechanisms. Although SIRT1 and SIRT6 both influence longevity, genomic stability and normal mitochondrial function, they may differ in their relationship with PARP1. SIRT6 is an upstream activator of PARP1,

whereas SIRT1 and PARP1 can mutually affect the activity of one another in response to a range of stimuli, including genotoxic, metabolic and circadian changes<sup>140</sup>. In addition, further mechanistic studies of DNA repair proteins that have nuclear and mitochondrial localization (BOX 1) are needed to extend our understanding of DNA damage-induced NM signalling.

Novel strategies to target different NM pathways and mitochondrial maintenance, such as sustaining energy metabolism, upregulating mitophagy and regulating DNA repair, should be explored in depth in experimental models with a view towards intervention in humans. For compounds targeting the NM pathways mentioned here (FIG. 5), some major questions remain. Do these compounds cross the blood–brain barrier? What are their optimal pharmacokinetic conditions? So far, indications are that certain compounds, such as nicotinamide riboside and ketones, may have little or no toxicity in humans, but this requires further exploration. This research area is ripe for development, and new mechanistic insights and intervention strategies will probably soon emerge from ongoing research.

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## Competing interests statement

The authors declare competing interests: see Web version for details.

## DATABASES

Mito DB (The mitochondrial disease database):  
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