BIO-GENAGE
Global Biohealth

MEDICAL ANALYSIS & RESEARCH FOR AGING & LONGEVITY SOLUTION

SIRTUIN SYSTEM 1-7 去氧核醣核酸-蛋白家族（沉默信息调节蛋白）GENE

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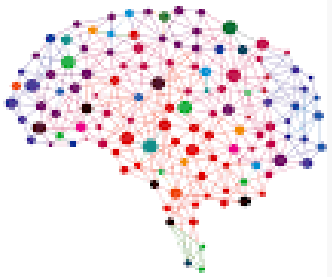

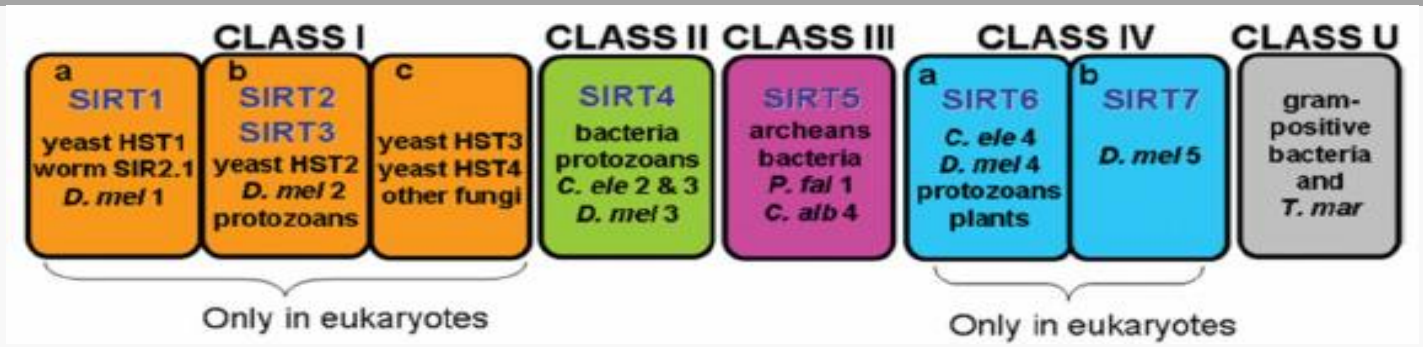

What are the sirtuins to do aging?

It is known now that sirtuins, when adjusting the pattern of cellular metabolism to nutrient availability, can regulate many metabolic functions significant from the standpoint of aging research – including DNA repair, genome stability, inflammatory response, apoptosis, cell cycle, and mitochondrial functions.

What are sirtuins and what are their effects on lifespan?

Sirt1 is reported to play a crucial role in metabolic homeostasis and IIS [61, 62]. AMPK signaling belongs to the protein kinase family and restores cellular energy levels. Increased AMPK activity is known to extend the lifespan of some model organisms.

How can I increase my sirtuins naturally?

There are several ways to stimulate the production of sirtuins, the longevity proteins. Examples are intermittent fasting or a simple reduction in daily calorie intake, which have long been linked to an increase in average life expectancy in several species.

What is the role of sirtuins?

Sirtuins are NAD+-dependent histone deacetylases regulating important metabolic pathways in prokaryotes and eukaryotes and are involved in many biological processes such as cell survival, senescence, proliferation, apoptosis, DNA repair, cell metabolism, and caloric restriction.

Where are sirtuins found?

Mammalian Sirtuins: Subcellular Localization. Mammalian sirtuins are found in numerous compartments within the cell (Table 1). SIRT1, -6, and -7 are found predominantly in the nucleus (59); SIRT3–5 reside in mitochondria; and SIRT2 is primarily cytoplasmic.

How many sirtuins are there?

In mammals, there are seven sirtuins, named SIRT1 to SIRT7. They are involved in a much broader range of cellular processes and pathways with distinct cellular localization and molecular targets.

Is sirtuin a gene or protein?

The sirtuins comprise a highly conserved family proteins present in virtually all species from bacteria to mammals. Sirtuins are members of the highly conserved class III histone deacetylases, and seven sirtuin genes (sirtuins 1–7) have been identified and characterized in mammals.

What are the 7 sirtuins?

Although only the SIRT1 isoform has been mentioned before, humans possess seven different sirtuins (SIRT1-7), which localize to several subcellular compartments such as the nucleus (SIRT1, 2, 3, 6, and 7), cytoplasm (SIRT1 and 2), and mitochondria (SIRT3, 4, and 5).

How can I increase my sirtuins naturally?

There are several ways to stimulate the production of sirtuins, the longevity proteins. Examples are intermittent fasting or a simple reduction in daily calorie intake, which have long been linked to an increase in average life expectancy in several species.

Does resveratrol actually activate SIRT1?

Recent biological studies have revealed that resveratrol does activate SIRT1 toward certain substrates containing a bulky hydrophobic group, such as a 7-amino-4-methylcoumarin (AMC) moiety or a tryptophan residue, directly adjacent to the acetylated Lys at the +1 position.

Is resveratrol a sirtuin?

Analysis into recent studies have revealed Resveratrol as a known activator of the protein deacetylase sirtuin 1 (SIRT1) gene, which is thought to mediate anti-proliferative and anti-inflammatory activity due to alteration of gene expression and modulation of numerous metabolic pathways.

The Sirtuin system: The Holy Grail of Resveratrol?

Research reference from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3613783/

Why Sirtuins are promising target in slowing down the ageing process?

Research reference from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5514220/

Slowing ageing by design: the rise of NAD+ and sirtuin-activating compounds

Research reference from https://www.nature.com/articles/nrm.2016.93

Why Sirtuin 7 is so important?

Sirtuin 7 (SIRT7), a mammalian Sir2 homolog, protects against rDNA instability and senescence in primary human cells.

Regeneration and Aging: Regulation by Sirtuins and the NAD+ Salvage Pathway

Research reference from https://www.sciencedirect.com/science/article/pii/B9780123809285100193

Sirtuins: The ‘magnificent seven’, function, metabolism and longevity

The sirtuin family of histone deacetylases (HDACs) was named after their homology to the Saccharomyces cerevisiae gene silent information regulator 2 (Sir2). In the yeast, Sir2 has been shown to mediate the effects of calorie restriction on the extension of life span and high levels of Sir2 activity promote longevity. **Like their yeast homologs, the mammalian sirtuins (SIRT1 - 7) are class III HDACs and require NAD+ as a cofactor to deacetylate substrates ranging from histones to transcriptional regulators.** Through this activity, sirtuins are shown to regulate important biological processes ranging from apoptosis, adipocyte and muscle differentiation, and energy expenditure to gluconeogenesis. We review here the current knowledge regarding the role of sirtuins in metabolism, longevity, and discuss the possible therapeutic applications that could result from the understanding of their function in different organs and pathologies.

What do sirtuins have to do with aging?

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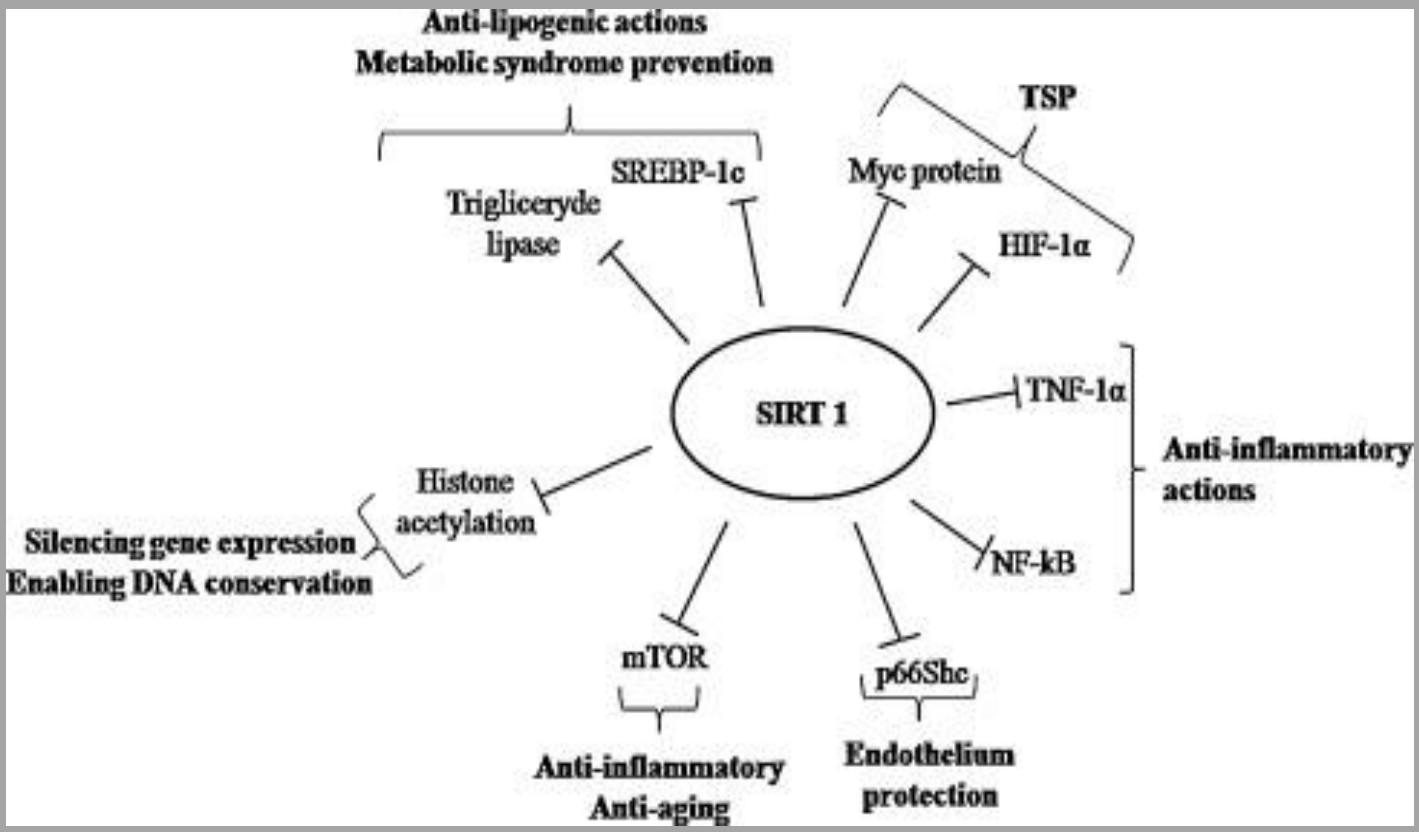


What foods have sirtuins?

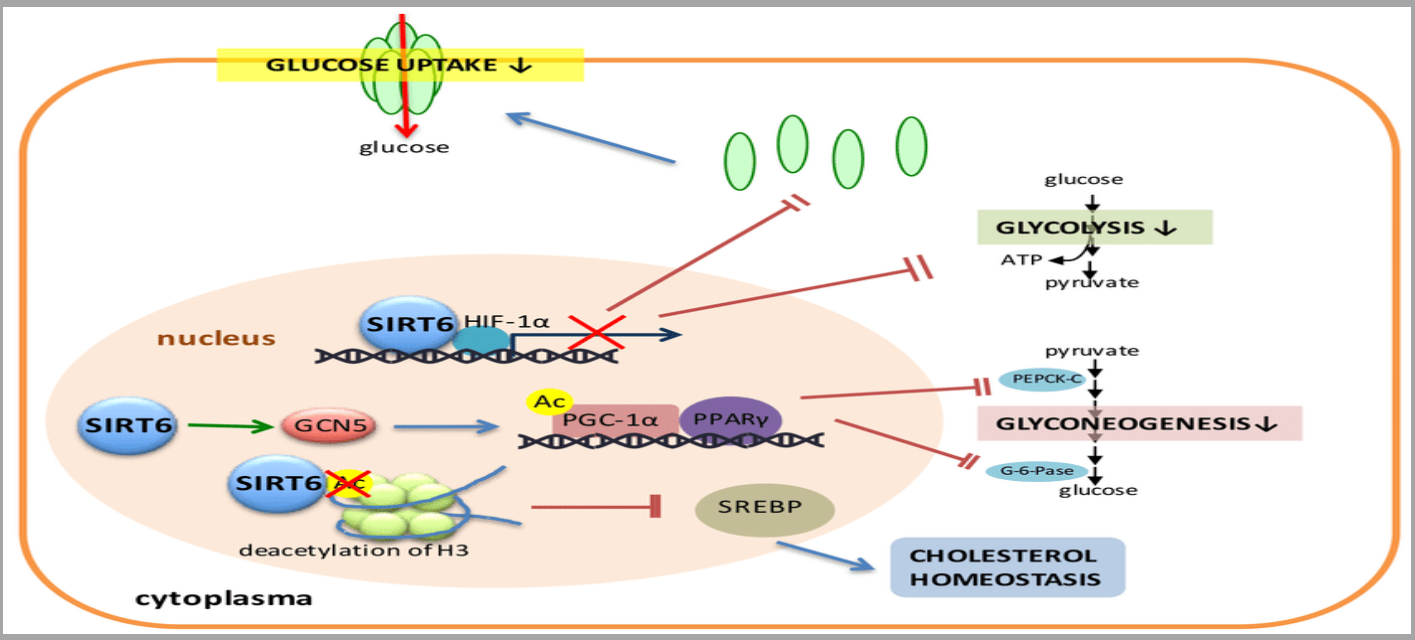
The Sirtfood Diet is based on research on sirtuins, a group of proteins that regulate several functions in the body.

What is the Sirtfood Diet?

1. kale.
2. red wine.
3. strawberries.
4. onions.
5. soy.
6. parsley.
7. extra virgin olive oil.
8. dark chocolate (85% cocoa)

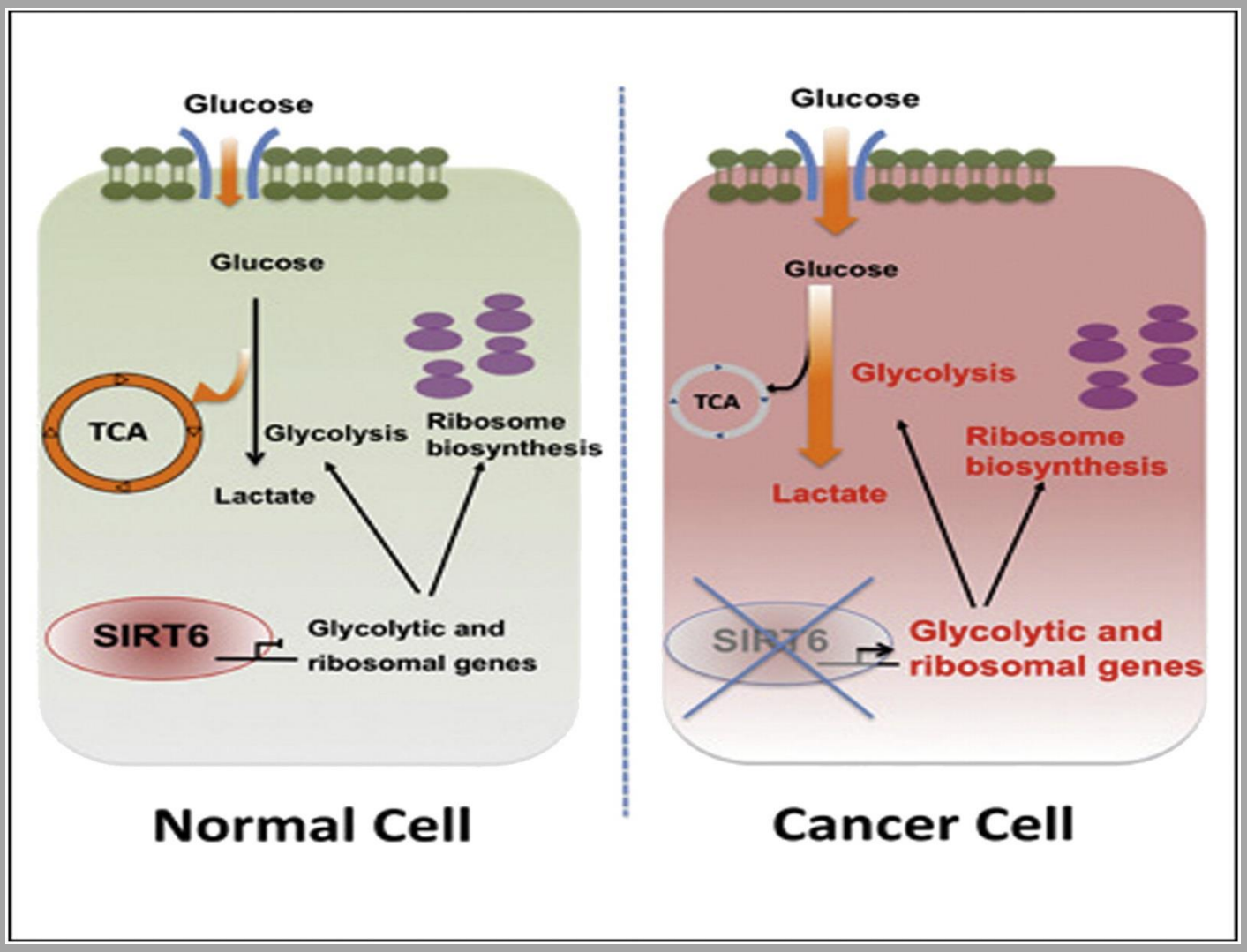
What Sirtuin 6 can functions?

Sirtuin 6 (SIRT6), one of the seven members of mammalian sirtuin family, localizes in the nucleus and primarily regulates chromatin signaling and genomic integrity. Recent studies established the critical role of SIRT6 in the pathophysiology of metabolic disease, as well as its roles in longevity and cancer. These roles that were determined by genetic studies include promoting pancreatic insulin secretion, inhibiting hepatic gluconeogenesis and triglyceride synthesis, and suppressing adiposity, suggesting that SIRT6 activators are promising molecules for treating obesity and diabetes. In contrast, a recent study showed that a synthetic inhibitor of SIRT6 improved glucose tolerance in a type 2 diabetes mouse model, associated with increased glycolysis and the expression of glucose transporter GLUT-1 and 4 in skeletal muscle, providing proof-of-concept evidence of SIRT6 inhibition as a treatment for diabetes. This review summarizes the confounding findings on the role of SIRT6 in metabolic homeostasis and discusses the possible relationships of these findings as they relate to the use of SIRT6 as a therapeutic target for type 2 diabetes and related diseases.



The Histone Deacetylase SIRT6 Is a Tumor Suppressor that Controls Cancer Metabolism

Importantly, loss of SIRT6 leads to tumor formation without activation of known oncogenes, whereas transformed SIRT6-deficient cells display increased glycolysis and tumor growth, suggesting that SIRT6 plays a role in both establishment and maintenance of cancer. By using a conditional SIRT6 allele, we show that SIRT6 deletion in vivo increases the number, size, and aggressiveness of tumors. SIRT6 also functions as a regulator of ribosome metabolism by core pressing MYC transcriptional activity. Lastly, Sirt6 is selectively downregulated in several human cancers, and expression levels of SIRT6 predict prognosis and tumor-free survival rates, highlighting SIRT6 as a critical modulator of cancer metabolism. Our studies reveal SIRT6 to be a potent tumor suppressor acting to suppress cancer metabolism.



Sirtuin 6 (SIRT6) extends mammalian lifespan and promotes healthspan.

SIRT6 maintains genomic stability through improved efficiency of DNA repair.

SIRT6 maintains heterochromatin and silences repetitive genetic elements.

Regulation of SIRT6 is a potential strategy for rejuvenation.

Sirtuin 6 (SIRT6) has been in the spotlight of aging research because progeroid phenotypes are associated with SIRT6 deficiency. SIRT6 has multiple molecular functions, including DNA repair and heterochromatin regulation, which position SIRT6 as a hub that regulates genome and epigenome stability. Genomic instability caused by persistent DNA damage and accumulating mutations, together with alterations in the epigenetic landscape and derepression of repetitive genetic elements, have emerged as mechanisms driving organismal aging. Enhanced levels of SIRT6 expression or activity provide avenues for rejuvenation strategies. This review focuses on the role of SIRT6 in the maintenance of genome and epigenome stability and its link to longevity. We propose a model where SIRT6 together with lamins control aging and rejuvenation by maintaining epigenetic silencing of repetitive elements.

Genomic and epigenomic instability drive the aging process

The complex etiology of aging is entwined with multiple overlapping pathological processes [1.]. To prevent pathology, each individual cell must maintain balanced homeostasis which relies on the maintenance of genomic stability and coordinated expression of specific sets of genes that are subject to epigenetic control. A variety of factors including environmental mutagens, cellular metabolism, oxidative stress, and genetic predisposition, can result in DNA damage that poses a major threat to genome and epigenome stability [2.]. Repeated cycles of DNA replication and persistent DNA damage throughout life may result in mutations, genomic rearrangements, loss of epigenome structure, and increased cell-to-cell variation in gene expression, thus driving aging phenotypes [3.]. Chromatin organization in eukaryotic cells provides epigenetic information that dictates cell identity. However, chromatin may undergo changes with advancing age, resulting in epigenetic drift (see Glossary) [4.,5.]. The accumulation of epigenetic changes leads to substantial cell-to-cell heterogeneity in gene expression, and this may further affect numerous signaling pathways that underlie the development of various hallmarks of aging [1.].

Gene expression at defined loci relies on dynamic regulation by epigenetic factors such as chromatin-modifying enzymes, which makes them master-regulators of cellular homeostasis. Therefore, chromatin modifiers may be key to understanding the pathological processes comprising aging and also present promising targets for longevity research. Members of the sirtuin family of proteins have been associated with aging because of their ability to influence multiple vital functions of a cell through the maintenance of genomic stability and control of gene expression [6.]. SIRT6 is one of the seven mammalian homologs of the silent information regulator 2 (Sir2) and is preferentially associated with chromatin [7.]. As a regulator of chromatin structure and DNA repair, SIRT6 is strategically positioned to maintain genomic and epigenomic stability, as well as influence gene expression, in multiple physiological and pathological pathways that determine longevity. This article reviews the role of SIRT6 in lifespan and health span regulation through the prism of its ability to control the cellular epigenetic landscape and genomic stability.

SIRT6 and lifespan in mammals

Because of early findings that Sir2 regulates lifespan in model organisms, SIRT6 has been investigated as a target for longevity research in mammals [8., 9., 10., 11., 12.]. Several groups have generated Sirt6 knockout (KO) mice and reported reduced lifespan in these animals, albeit the severity of the phenotype seems to depend on the genetic background. Sirt6 KO mice on a 129/SvJ background die soon after weaning and do not survive longer than 4 weeks, exhibiting a progeroid phenotype [7.,13.,14.]. In animals with a mixed genetic background (129/Black Swiss/FVB), ~60% of Sirt6 KO mice also die before reaching 1 month of age; however, >75% of the female and 10% of male mice survive past 300 days [15.]. Sirt6 KO in mice leads to reduced body weight, low blood glucose levels, lymphopenia, and severe hypoglycemia, acute loss of subcutaneous fat, lord kyphosis, and degenerative signs in bone and colonic epithelium, indicating multiple tissue homeostasis failures [7.]. Studies in global Sirt6 KO and various cell type-specific Sirt6 KO models have revealed the contribution of SIRT6 deficiency to vascular aging, neurodegeneration, retinal dysfunction, stroke, muscle dystrophy, osteoporosis, obesity, fibrosis, and β-cell dysfunction [16.]. An even more severe phenotype is caused by SIRT6 deficiency in primates: when SIRT6 was knocked out in monkeys only females survived to birth and

died within days [17.]. Likewise, an inactivating homozygous mutation D63H in human SIRT6 was found to cause perinatal lethality, and SIRT6 deficiency resulted in sex reversal in the male fetus [18.]. A recent study identified eight mutations in SIRT6 in melanoma patients, four of which correlated with high mutation rates across the genome [19.]. A strong negative correlation between SIRT6 protein expression and age has been found in human primary skin fibroblasts [20.]. Reduced expression of SIRT6 was detected in the hippocampus, buccal epithelium, and peripheral blood mononuclear cells (PBMCs) of elderly patients [21., 22., 23.]. Decreased SIRT6 levels have been associated with several human age-related pathologies, including some cancers, cardiovascular diseases, atherosclerosis and hypertension, liver fibrosis, Alzheimer's disease, metabolic diseases, and others [16.,24., 25., 26., 27., 28., 29.].

Although gene knockouts often lead to various pathologies and shortened lifespan in mice, the most remarkable feature of SIRT6 is that its overexpression extends mammalian lifespan. In a recent study Roichman et al. discovered an impressive 27% increase in median lifespan for male mice compared to wild-type littermates, and 15% for female mice, when SIRT6 was overexpressed under a CAG promoter [30.]. Moreover, SIRT6 overexpression improved various aspects of organismal homeostasis and promoted health span at multiple levels [31.]. One of the important aspects involving SIRT6 in longevity is its remarkable ability to influence metabolic pathways. SIRT6 deficiency impairs hepatic ability to perform β-oxidation [32.]. SIRT6 overexpression promotes β-oxidation in liver, hepatic lactate and glycerol shuttling, improves the NAD+/NADH ratio, and stimulates glycerol release from adipose tissue. In addition, overexpression of SIRT6 improves the capacity to produce glucose, which decreases with age [30.]. Metabolic regulation is intimately connected to the development of cancer, which becomes more prevalent with age. Interestingly, SIRT6 overexpression leads to a decrease in the incidence of neoplasms in aged mice [30.]. This effect is likely to result from both improved genome stability and SIRT6-mediated transcriptional regulation of metabolic processes, such as repression of glycolysis and suppression of the Warburg effect [24.,33.], thus linking epigenetics, regulation of metabolism, cancer, and aging. The positive effects of SIRT6 on healthspan are not limited to metabolic regulation because SIRT6 overexpression alleviates endothelial cell dysfunction, improves adult neurogenesis, decreases infarct size and neurological deficit in a stroke model, protects from kidney injury and colitis, and helps to maintain genomic stability in the brain [25.,29.,34., 35., 36., 37., 38., 39.]. Intriguingly, genetic polymorphisms in SIRT6 have been found to be associated with human longevity; however, the role these polymorphisms play in SIRT6 expression and function remains to be elucidated [40., 41., 42., 43.].

Taken together, there is strong evidence that SIRT6 deficiency shortens lifespan and leads to the development of pathologies in a variety of tissues. High levels of SIRT6 help to rescue these severe phenotypes in experimental models, implying that maintenance of stable or increased SIRT6 expression and activity throughout life could help to counteract aging and positively influence healthspan.

SIRT6 structure and its enzymatic activities

SIRT6 is predominantly associated with the chromatin fraction of cell nuclei. SIRT6 can also be observed in the cytoplasm localized to stress granules, where it may be an active component that facilitates their assembly [44.,45.]. The crystal structure of SIRT6 reveals a conserved globular catalytic core domain comprising 275 amino acids with a Rossman-fold and a Zn2+-binding domain, a less well conserved N terminus, and a non-conserved and highly disordered C terminus (Figure 1) [46.]. The N terminus, that is rich in positively charged residues, is crucial for chromatin association and histone deacetylation in vitro, whereas the highly disordered C terminus contains a nuclear localization signal and is responsible for the differences in the strength of SIRT6 enzymatic activity across species with various lifespans [47.,48.].

The core domain of SIRT6 utilizes NAD+ to provide two main enzymatic functions that influence chromatin states – deacylation and mono-ADP-ribosylation activities [44.,49.]. The most notable special case of deacylation is deacetylation and, as a nuclear sirtuin, SIRT6 has been classified as class III histone deacetylase that removes acetyl groups from the amino group of lysines and produces nicotinamide and O-acetyl-ADP-ribose as byproducts [49.]. SIRT6 demonstrates only weak deacetylation activity in vitro; however, SIRT6 overexpression in vivo leads to a strong reduction in the global

levels of H3 acetylation. This apparent discrepancy could be resolved by at least two observations: the efficiency of deacetylation has been demonstrated to depend on complex interactions with nucleosomes, and the deacetylation activity of SIRT6 can be significantly improved upon binding of fatty acids, which results in conformation changes that facilitate binding to acetylated H3 [50.,51.]. The major histone substrates for deacetylation by SIRT6 include histone H3 lysine 9 (H3K9), H3K56, and H3K18, through which SIRT6 can directly or indirectly control the promoters of many important genes, most notably transcription factors [52., 53., 54.]. Another important mechanism for gene regulation by SIRT6 occurs in intragenic regions at the level of transcription, whereby SIRT6 binding to RNA polymerase II (Pol II) stabilizes Pol II promoter-proximal pausing and inhibits transcriptional elongation [55.].

SIRT6 is also capable of removing longer fatty acyl groups owing to the presence of the elongated hydrophobic pocket in its structure, which ensures a hundred-fold higher catalytic activity toward long-chain peptide substrates compared to acetylated substrates in vitro [56.]. This feature allows it to mediate efficient deacylation of H3K9, H3K18, H3K27, and to a lesser extent H3K14, H3K36, H3K56, and H3K79 [57.]. Apart from histone deacylation, SIRT6 has many important non-histone targets, such as tumor protein p53, histone acetyltransferase GCN5, tumor necrosis factor (TNF)-α, and nuclear factor erythroid 2-related factor 2 (NRF2). The SIRT6-mediated removal of long-chain acyl groups from lysines K19 and K20 of TNF-α is particularly interesting because this has been shown to promote the secretion of this important inflammatory cytokine [58.,59.]. SIRT6 also acts as an ADP-ribosytransferase to mono-ADP-ribosylate nuclear proteins with the production of nicotinamide [44.]. This activity of SIRT6 is less well studied because of the difficulties associated with detection of mono-ADP ribosylation. SIRT6 actively ribosylates itself in vivo and in vitro, but the function of self-ribosylation is unknown [44.]. The known trans substrates of SIRT6 include poly(ADP-ribose) polymerase 1 (PARP1), KRAB-associated protein 1 (KAP1), BRG1-associated factor 170 (BAF170), and lysine-specific demethylase 2A (KDM2A), which mediate a wide range of cellular functions such as DNA repair, heterochromatin relaxation and compaction, and antioxidant defense [60., 61., 62., 63.]. Armed with these powerful tools, SIRT6 is well equipped to influence a variety of physiological and pathological pathways related to organismal and cellular aging, most notably DNA repair and epigenome maintenance.

SIRT6 and DNA repair

SIRT6 has been implicated in DNA repair of both single-strand breaks (SSBs) and double-strand breaks (DSBs). Sirt6 KO mice exhibit chromosomal abnormalities indicative of DNA repair defects [7.]. The proper repair of DSBs is particularly important to counteract aging. DNA DSB repair efficiency declines with age and becomes compromised during replicative senescence in mammalian cells [64.]. As mammalian fibroblasts approach the senescent state, they express lower SIRT6 levels compared to cells at lower population doublings. Overexpression of SIRT6 rescues the DSB repair efficiency in senescent cells and stimulates the efficiency of both homologous recombination (HR) and non-homologous end joining (NHEJ) DSB repair pathways, as evidenced by chromosomally integrated reporter assays [60.,64.].

Mammalian maximum lifespans (MLS) differ up to 100-fold between species [65.], and species longevity has been shown to correlate with the efficiency of DSB repair by NHEJ and HR repair pathways [48.]. Remarkably, SIRT6 was shown to be the major factor responsible for the correlation between longevity and DSB repair. SIRT6 from long-lived species has stronger deacetylation and mono-ADP-ribosylation activities, and serves as a more potent activator of DSB repair. Thus, animals with the longest lifespan exhibit not only high expression of DSB repair genes but may also have evolved unique alleles of key aging genes that allow them to avoid, delay, or alleviate the hallmarks of aging [66.,67.].

SIRT6 has been implicated in multiple stages of DSB repair. SIRT6 is rapidly recruited to DNA DSB sites [60.,68.,69.]. It was recently reported that SIRT6 can recognize and directly bind to the damaged DNA, serving as a DNA break sensor [70.,71.]. In addition, SIRT6 has been shown to anchor to the phosphorylated histone H2A variant, γH2AX [70.]. Upon DSB damage, H2AX is rapidly phosphorylated by ataxia-telangiectasia mutated (ATM) kinase, which further recruits and localizes repair proteins near to the DNA breaks. Interestingly, deacetylation of SIRT6 at residue K33 by SIRT1 is required

for the interaction between SIRT6 and γH2AX, revealing a synergy between the two sirtuins. In addition, the radiation-induced H2AX-interacting protein Bcl-2-associated transcription factor 1 (BCLAF1) has been reported to be a binding partner of SIRT6 [72.].

A direct interaction has been shown between SIRT6 and Ku80, an abundant ring-shaped molecule that tightly associates with DSBs and initiates the assembly of the NHEJ machinery upon formation of a DSB [73.,74.]. The chromatin-associated endogenous SIRT6 interacts with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and stabilizes it at the sites of DNA breaks [73.]. Moreover, SIRT6 binding to Ku80 promotes phosphorylation of DNA-PKcs at S2056, resulting in an improved efficiency of NHEJ repair [74.].

SIRT6 is particularly relevant under oxidative stress (OS) conditions, which may be the source of persistent DNA damage in aged tissues. Inhibitors of various stress-activated kinases were screened on human skin fibroblast cells under OS conditions, and the c-Jun N-terminal kinase (JNK) pathway has been identified as a regulator of the ability of SIRT6 to stimulate DSB repair via phosphorylation of SIRT6 on S10 [69.]. Upon its recruitment to DSBs under OS, SIRT6 mono-ADP-ribosylates PARP1 on K521, thereby stimulating its poly-ADP-ribosylase activity and enhancing DSB repair by relaxing chromatin and facilitating the recruitment of DNA repair factors such as Rad51, NBS1, and 53BP1 [60.,64.].

The repair of SSB DNA lesions created by endogenous alkylation, oxidation, and deamination as a result of OS also deserves attention in the context of aging. A strong case has been made for SIRT6 involvement in SSB repair through the base-excision repair (BER) pathway, supported by direct association between SIRT6 and core BER factors [7.,75.,76.]. During damage sensing in BER, SIRT6 associates with the MYH/MUTYH glycosylase, heterotrimeric 9-1-1 checkpoint clamp (RAD9, RAD1, and HUS1 proteins), and apurinic/apyrimidinic endonuclease 1 (APE1), and promotes their activities in response to OS [75.]. Furthermore, Xu et al. showed that BER efficiency declines as a function of age in human skin fibroblasts from donors of different ages, and overexpression of exogenous SIRT6 rescued the decline of BER in these aged fibroblasts [20.]. SIRT6 is also involved in the nucleotide excision repair (NER) pathway that is responsible for the removal of bulky DNA adducts induced by UV irradiation or genotoxic chemicals. Lack of SIRT6 led to reduced NER efficiency based on the GFP plasmid reactivation assay following UV light exposure, whereas overexpression of SIRT6 promoted NER repair [19.]. SIRT6 deacetylation of DNA damage-binding protein (DDB2) has been shown, which promotes the ubiquitination of DDB2 and segregation of DDB2 from chromatin, thus facilitating NER signal transduction [19.]. Unlike BER, defects in the NER pathway can lead to several human syndromes that are associated with shortened lifespan, such as xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy.

DNA repair in eukaryotes requires chromatin remodeling in the areas of damaged DNA to allow access of the DNA repair machinery to the damaged sites. SIRT6 cooperates with several other factors to shape the local chromatin environment, and lack of SIRT6 has been associated with impaired chromatin remodeling and increased sensitivity to genotoxic damage. As the activator of PARP1, SIRT6 may be crucially involved in chromatin remodeling in response to DNA damage because PARP1 rapidly poly-ADP-ribosylates (PARylates) chromatin and mediates initial chromatin relaxation through recruitment of chromatin remodeling complexes such as NuRD [60.,77.]. Furthermore, SIRT6 deacetylation of H3K9 is a prerequisite for PARP1 ribosylation of H3S10 during DNA repair [78.]. Recently an interaction between SIRT6 and a subunit of NuRD, chromodomain helicase DNA-binding protein 4 (CHD4), has been shown in response to DNA damage, leading to displacement of heterochromatin protein (HP) 1 by CHD4 and promoting chromatin relaxation for HR-mediated repair [79.]. Furthermore, SIRT6 deacetylates H3K56 at DSBs and recruits the chromatin remodeling factor SNF2H to the damage site, which in turn promotes local chromatin accessibility [68.]. SIRT6 has also been shown to deacetylate histone acetyltransferase (HAT) GCN5 at K549, thereby enhancing its activity, and this may further lead to chromatin accommodation for repair [80.]. When DNA damage occurs in actively transcribed regions, alterations in euchromatin structure help to halt local transcription in transient manner to allow repair to proceed. Mass spectrometry followed by mutagenesis analysis showed that lysine-specific demethylase 2A (KDM2A) is mono-ADP-ribosylated by

SIRT6, resulting in displacement of KDM2A from the DSB location, leading to enrichment of histone H3 di- or trimethylated on lysine 36 (H3K36me2/3) marks and enhancing NHEJ repair efficiency [63.].

At the final stage of DNA repair the original structure of chromatin must be restored to prevent the accumulation of aberrantly packaged chromatin which may promote tissue aging. The H3K9me3-specific histone methyltransferase SUV39H1 has been shown to be a binding partner of SIRT6 [81.]. Methyltransferases create an initial nucleation event on the euchromatin, followed by cycles of H3K9 methylation and loading of new HP1α complexes leading to spreading of nascent heterochromatin. It is tempting to speculate that, via interactions with SUV39H1 and possibly other chromatin factors, SIRT6 may not only regulate transcription but also participate in restoring the original heterochromatin structure following DNA damage.

In summary, SIRT6 plays important roles in regulating different stages of DNA repair. SIRT6 main functions in DNA repair involve facilitating chromatin remodeling and the orderly recruitment of repair enzymes to sites of DNA damage, acting as an upstream regulator that promotes both the HR and NHEJ repair pathways

SIRT6 at different stages of DNA repair.

Altering the expression or activity of DNA repair enzymes has given disappointing results and has not resulted in improved DNA repair efficiency, probably because of miscoordination and imbalance between repair enzymes. SIRT6 overexpression, by contrast, enhances multiple pathways of DNA repair owing to its upstream regulatory role. The regulatory role of SIRT6 in DNA repair suggests that SIRT6 is a promising target for antiaging interventions and enhancement of DNA genome stability.

SIRT6 and epigenome stability

Persistent DNA damage with advancing age may result in gradual changes in chromatin structure and erosion of the epigenetic landscape, which may be particularly harmful in regions of constitutive heterochromatin. SIRT6 has been shown to colocalize with HP1β within the nucleus, which potentially implicates it in aging-related epigenetic alterations specifically in regions of constitutive heterochromatin at repetitive DNA sequences, including pericentromeric and telomeric regions, retrotransposable elements, and lamina-associated domains (LADs) [60.].

Telomeres in actively dividing cells are subject to shortening following successive rounds of DNA replication, which may lead to genomic instability, cellular senescence, and abnormal gene expression in subtelomeric regions. Cells lacking SIRT6 display telomere dysfunction and chromosomal abnormalities reminiscent of the premature aging disorder Werner syndrome, whereas overexpression of SIRT6 improves telomere integrity through H3K9 deacetylation and increased association of WRN with chromatin [82.,83.]. The cell cycle-dependent regulation of H3K56 acetylation by SIRT6 is important for the maintenance of telomeric chromatin [53.,84.]. SIRT6 has also been shown to directly target the telomere repeat protein (TRF) 2, which undergoes ubiquitin-mediated proteolysis upon deacetylation by SIRT6 [85.]. Another important function of telomeres is to repress nearby gene expression through telomere position effect (TPE). SIRT6 deficiency may lead to derepression of subtelomeric regions, that are rich in H3K9me3/SUV39H/HP1, as shown by increased expression of a chromosomally integrated reporter at a telomere [86.]. Telomeres are also highly susceptible to oxidative damage which accelerates telomere shortening. Upon oxidative damage, MYH foci are formed at telomeres and SIRT6 is recruited to the DNA damage site to aid in repair [76.].

SIRT6 has also been found to colocalize with the marker of centromeres, CENP-A [54.]. SIRT6 deficiency is associated with aberrant expression of centromeric satellite sequences and SIRT6 is required for deacetylation of H3K18, which promotes centromeric silencing [54.]. Pericentromeric heterochromatin domains have a major influence on chromosome

segregation and stability, and knockdown of SIRT6 leads to an impairment of chromosome segregation, implicating SIRT6 in the regulation of mitosis

SIRT6 and epigenome maintenance.

Aging-related reorganization of heterochromatin leads to derepression of transposable elements – genomic parasites that account for almost a half of the human genome and lead to genomic instability during aging [90.]. Type 1 long interspersed nuclear elements (LINE1s) are retrotransposable elements which are particularly relevant to aging because their activity is elevated in aged somatic tissues [14.,91.]. Activation of LINE1 elements leads to the accumulation of LINE1 cytoplasmic DNA copies that can be detected by the DNA sensor cyclic GMP-AMP (cGAMP) synthase (cGAS), which triggers an innate immune response by activating the stimulator of interferon response cGAMP interactor (STING) 1 to produce interferon (IFN) type I, thus promoting sterile inflammation [14.]. Sirt6 KO mice show a dramatic increase in LINE1 cDNA – comparable to or higher than in old wild-type mice. Conversely, overexpression of SIRT6 in mice represses LINE1 activity [14.,92.]. The mechanism of LINE1 repression by SIRT6 relies on mono-ADP-ribosylation of KAP1, which is involved in the maintenance of the epigenetically stable heterochromatin [61.]. Modification of KAP1 by SIRT6 at LINE1 loci promotes KAP1 complex formation with HP1, thereby packaging LINE1 DNA into transcriptionally silent heterochromatin [61.].

In summary, SIRT6 functions to maintain the heterochromatin state of a wide range of genetic elements including telomeres, centromeres, and transposable elements (Figure 3). Loss of silencing of these elements is associated with aging and age-related disease.

Rejuvenation through reprogramming involving SIRT6

Rejuvenation of the aged epigenetic landscape could be achieved by reprogramming. Complete reprogramming of somatic cells can be performed by conversion into induced pluripotent stem cells (iPSCs) via overexpression of the Yamanaka factors OCT4, SOX2, KLF4, and MYC (OSKM) [93.]. SIRT6 deficiency decreased the reprogramming efficiency in mouse cells, and SIRT6 was found to improve the reprogramming efficiency of human dermal fibroblasts [94.,95.]. The SIRT6 activator MDL-800 improved the pluripotency of murine iPSCs [96.]. Moreover, when a combination of SIRT6 and Yamanaka factors was used, NHEJ DSB repair was improved in iPSCs derived from old mice [74.]. In embryonic stem cells (ESCs), SIRT6 histone deacetylation activity has been shown to play a crucial role in stem cell fate determination through the repression of the key pluripotency factors OCT4, SOX2, and NANOG [55.]. Complete reprogramming is undesirable for in vivo rejuvenation because iPSCs may be tumorigenic. As a potential solution to this problem, partial reprogramming could be employed, and this has been shown to have a rejuvenating effect in mouse models [97.,98.]. It is tempting to speculate that the addition of SIRT6 may facilitate safer reprogramming by returning chromatin to its more youthful conformation.

SIRT6 cooperates with LMNA to maintain a youthful epigenome

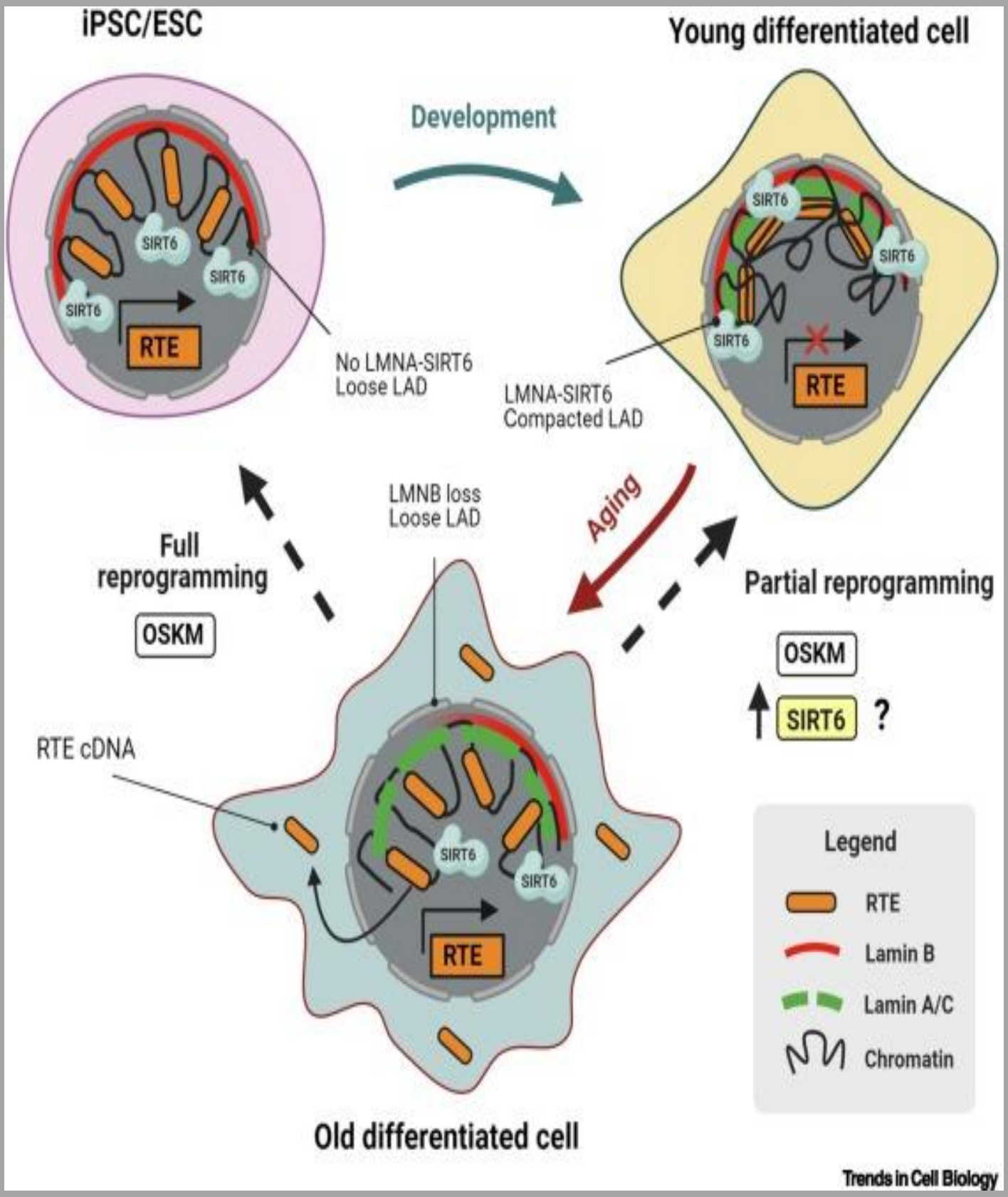
Lamin A/C (LMNA) is a nuclear scaffold protein that mediates chromatin packaging [99.]. LMNA binds to the regions of LAD heterochromatin and tethers them to the nuclear periphery. LADs are enriched in retrotransposable elements, which can become derepressed in aged cells. LMNA mutations are associated with several genetic syndromes including the premature aging disease Hutchison–Gilford progeria syndrome (HGPS) [100.]. Remarkably, polymorphisms in LMNA are also found in centenarians, indicating that LMNA variants may promote longevity [100.]. LMNA helps to establish repressive heterochromatin at the nuclear periphery, but in the cells from HGPS patients a global loss of chromatin compartmentalization is observed [101.]. LMNA is linked to epigenetic reprogramming and pluripotency. LMNA is not expressed, or expressed at very low levels, in embryonic cells and iPSCs, but is turned on with cell differentiation [102.,103.]. Reprogramming of HGPS cells into iPSCs rescues their phenotype, which then reappears upon differentiation

[104.]. LMNA directly interacts with the SIRT6 catalytic core domain via its C terminus and promotes SIRT6 deacetylation and mono-ADP-ribosylation activities, acting as an endogenous activator of SIRT6 [105.]. Based on the interaction between SIRT6 and LMNA, and the central role of SIRT6 in maintaining heterochromatin organization of repetitive sequences and retrotransposable elements, we propose the following model: the SIRT6–LMNA axis may control epigenetic aging and the rate of epigenetic drift (Figure 4). In a young somatic cell, SIRT6–LMNA maintain heterochromatin, which keeps retrotransposons in a packed and silent state. The LADs and retrotransposons are tethered to the nuclear periphery, thus maintaining a youthful transcriptional pattern and differentiated state of the cells. During aging, with multiple rounds of replication, transcription, and DNA repair, heterochromatin unravels, leading to derepression of retrotransposons, the accumulation of cytoplasmic retrotransposon copies, and ultimately the induction of sterile inflammation and functional decline. Upon full reprogramming, LMNA expression is turned off and the epigenome becomes permissive of retrotransposon expression. Partial reprogramming would not remove LMNA but instead 'tightens up' repetitive elements into heterochromatin, thus rejuvenating the epigenome. We hypothesize that enhanced expression or activity of SIRT6, alone, or coupled to partial reprogramming, may be able to reverse these age-related changes in heterochromatin and rejuvenate the epigenome.

Packaging of retrotransposable elements into heterochromatin controls aging and rejuvenation.

The benefits observed in SIRT6-overexpression animal models, and the pathologies that are associated with SIRT6 deficiency, suggest that activating SIRT6 may be a potential rejuvenating strategy. Like other sirtuins, SIRT6 expression can be increased by calorie restriction – one of the most robust and long-known mechanisms for life extension [106.]. A search for chemical SIRT6 activators revealed that SIRT6 can be activated in vitro through interactions with fatty acids such as myristic acid. Binding of a long-chain acyl group in the hydrophobic pocket of SIRT6 results in enhanced SIRT6 deacetylase activity [51.]. Taking advantage of such interactions, a screen of 432 lipid compounds for their ability to stimulate SIRT6 deacetylation of H3K9ac peptide in vitro identified novel compounds with the ability to increase SIRT6 activity by up to 48-fold [107.]. Several potent synthetic activators of SIRT6 deacetylase activity have been recently identified. These include an allosteric SIRT6 modulator MDL-800 that was shown to be effective in promoting SIRT6-dependent cell-cycle arrest and inhibiting tumor growth in vivo, as well as promoting genomic stability by activating DNA repair pathways [96.,108.,109.]. Another synthetic activator of SIRT6 deacetylation, UBCS039, has been shown to promote autophagy in a SIRT6-dependent manner [110.]. In addition, several natural compounds have shown promise as activators of SIRT6. A polyphenol cyanidin produced a 55-fold increase in SIRT6 activity in vitro [111.]. A plant flavone derivative, quercetin, could also activate SIRT6 deacetylation activity [112.]. Recently, SIRT6 transcription was found to be promoted by the alkaloid licorine, and this was associated with increased NHEJ and HR repair efficiency in human fibroblasts [113.]. Moreover, a polysaccharide isolated from brown algae, fucoidan, has shown robust SIRT6 activation in vitro and has been linked Concluding about Aging is associated with genomic instability

Aging is associated with genomic instability and reorganization of the epigenetic landscape, which are intimately linked together. SIRT6 operates at the crossroads between these processes and regulates numerous pathological pathways, working to preserve healthspan. It is possible that SIRT6 levels or activity decrease during aging, albeit to a different degree in various tissues and cells. Looking forward, targeted restoration of SIRT6 levels or stimulation of SIRT6 activity in cells in need could help to preserve their youthful phenotype. Many important questions remain unexplored, such as the effects of SIRT6 overexpression on DNA methylation profile, epigenetic clock [118.], and chromatin compaction in aged tissues. Ongoing research on SIRT6 in long-living species of mammals also holds promise to discover novel and more efficient SIRT6 variants. The functions of SIRT6 in DNA repair, the maintenance of heterochromatin domains, repression of transposable elements, and regulation of reprogramming provide exciting opportunities for novel rejuvenation strategies to fight age-related pathologies in humans (see Outstanding questions).


iPSC/ESC
Young differentiated cell
Development
SIRT6
SIRT6
SIRT6
RTE
No LMNA-SIRT6
Loose LAD
LMNA-SIRT6
Compacted LAD
LMNB loss
Loose LAD
Aging
Full
reprogramming
OSKM
Partial reprogramming
OSKM
SIRT6
?
RTE cDNA
Legend
RTE
Lamin B
Lamin A/C
Chromatin
Old differentiated cell
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