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Sirtuins as regulators of metabolism and healthspan

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Preface

Since the beginning of the century, the mammalian sirtuin protein family (SIRT1–SIRT7), has received much attention for their regulatory role, mainly in metabolism and aging. Sirtuins act in different cellular compartments: they deacetylate histones and several transcriptional regulators in the nucleus, but also specific proteins in other cellular compartments, such as in the cytoplasm and in mitochondria. As a consequence, sirtuins regulate fat and glucose metabolism in response to physiological changes in energy levels, thereby acting as crucial regulators of the network that controls energy homeostasis, and as such determines healthspan.

Keywords

Aging; Longevity; Metabolic disease; Mitochondria; Resveratrol; Sirtuins

Metabolic control involves a delicate balance between energy intake, utilization and storage. When food is ample, the excess energy is stored so that it can be used in times of scarcity. A carefully tuned regulatory and evolutionary conserved programme controls these switches in nutrient intake, use and storage, involving classical food excess signaling pathways, such as those revolving around insulin/IGF1 and target of rapamycin (TOR; mTOR in mammals), and food restriction pathways involving AMP-activated protein kinase (AMPK) and sirtuins (for further reading on these pathways, we refer the reader to refs1–4).

Sirtuins have received significant attention since the discovery that the yeast sirtuin silent information regulator 2 (Sir2), which was originally described as a regulator of transcriptional silencing of mating-type loci, telomeres and ribosomal DNA (reviewed in 5,6), extends yeast lifespan7. As Sir2 was soon discovered to be an NAD-dependent histone deacetylase8, it became apparent that sirtuins serve both as energy sensors and as transcriptional effectors by controlling the acetylation state of histones. What is more, sirtuins do not just deacetylate histones, but also a wide range of transcriptional regulators, thereby controlling their activity.

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In mammals the sirtuin family comprises seven proteins (SIRT1-SIRT7), which vary in tissue specificity, subcellular localization, enzymatic activity and targets. Sirtuins, notably SIRT1, have been studied for their role in caloric restriction (the only physiological intervention that extends lifespan), the prevention of aging-related diseases and the maintenance of metabolic homeostasis. As a consequence, the hunt for nutriceutical (obtained through food) or pharmaceutical sirtuin activators was intense and led to the identification of several sirtuin activator compounds. Especially resveratrol, a polyphenol found in red grapes, berries and peanuts, received a lot of attention9. Activation of sirtuins is thought to be beneficial not only for diseases relating to metabolism, such as type 2 diabetes and obesity, but also for neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. This is in part because sirtuins stimulate the activity of mitochondria, the powerhouses of the cell, and of mitochondrial proteins, which have a key role in the above-mentioned pathologies.

Here we present our current knowledge of the sirtuin family, discussing their mode of action, function and regulation. We focus primarily on SIRT1, as it is the best described sirtuin, and place particular focus on the impact of sirtuins on metabolic homeostasis and healthspan.

The sirtuin family

Sequence-based phylogenetic analysis revealed that mammalian sirtuins can be divided in four classes: SIRT1-SIRT3 belong to class I, SIRT4 to class II, SIRT5 to III, and SIRT6 and SIRT7 to class IV10. Below we discuss the subcellular localization of sirtuins, their mode of action and their functions in different compartments.

Sirtuin subcellular localization

Mammalian sirtuins show a discrete pattern of subcellular localization. SIRT1 is mainly localized in the nucleus, but also is present in the cytosol. Its nuclear export signal allows shuttling to the cytosol under specific circumstances, for instance when the insulin pathway is pharmacologically inhibited11. Although the physiological relevance of this shuttling is unclear, one can imagine that either cytosolic targets could be deacetylated, or that shuttling is another level of control on nuclear target proteins. SIRT2 is considered to be cytosolic, but is also present in the nucleus in the G2/M cell cycle transition12. SIRT3, SIRT4, and SIRT5 all have a mitochondrial targeting sequence, and their localization to this organelle has been confirmed experimentally13. SIRT6 is predominantly nuclear14, and SIRT7 was reported to reside in the nucleolus15, but further research is necessary to confirm this localization and its physiological relevance.

Sirtuin enzymatic activity

Originally, sirtuins were described as NAD-dependent type III histone deacetylases (HDACs), as the founding sirtuin — Sir2 in yeast — silenced specific genomic loci by deacetylating histone H3 and H416. Interestingly, mammalian sirtuins — notably those in class I — do not only target histones, but also deacetylate a wide array of proteins in different subcellular compartments (see above; Box 1). In addition, SIRT417 and SIRT618 were reported to function as ADP-ribosyltransferases, even though SIRT6 also can act as a

deacetylase19,20. SIRT5 was initially reported to deacetylate the urea cycle enzyme carbamoyl phosphate synthetase 1 (CPS1)21, but was recently shown to primarily demalonylate and desuccinylate proteins22, including CPS123 (Box 1).

The enzymatic reaction catalyzed by sirtuin proteins requires NAD^+ as a substrate, which is converted to nicotinamide, and the concentration of which is determined by the nutritional state of the cell24. As such, NAD^+ is well positioned to control adaptive responses to energy stress by modulating the activity of sirtuins and their downstream effectors, as will be discussed below.

During the sirtuin enzymatic reaction, nicotinamide is formed, which at higher concentrations can non-competitively bind and thereby feedback-inhibit sirtuin activity25,26. The other by-product of the sirtuin deacetylase reaction — O-acetyl-ADP-ribose — was also reported to be a signaling molecule27,28, but similarly to nicotinamide, its exact role in metabolic control warrants further investigation (for details see 24,29).

Sirtuin function — SIRT1

The most-studied member of the mammalian sirtuin family is SIRT1, which was originally described to deacetylate histones, but soon after also shown to deacetylate other protein targets (Table 1)(see 5,30,31 for more details). The first-described non-histone target for SIRT1 was p53, which is deacetylated and repressed upon DNA damage or oxidative stress, resulting in impaired apoptosis32,33. It was therefore hypothesized that increased SIRT1 activity could be tumorigenic, but the contrary seems to be the case (reviewed in 34). The activity of PGC1α, a transcriptional coregulator that governs mitochondrial biogenesis and activity, is also controlled by reversible acetylation35,36. PGC1α deacetylation by SIRT1 leads to its activation and the induction of downstream pathways that control mitochondrial gene expression37–42. Similarly, SIRT1 controls the acetylation of FOXO transcription factors, which are important regulators of lipid and glucose metabolism as well as stress response (see below). It is thought that SIRT1-mediated deacetylation does not just activate or inhibit FOXO, but it selectively directs FOXO to certain targets, thereby conferring another layer of specificity in addition to regulation by phosphorylation (see below)43–45.

Sirtuin function — SIRT3

Three sirtuins localize primarily to the mitochondria—SIRT3-SIRT5 (for recent reviews see 46,47). Of these, SIRT3 is the major mitochondrial deacetylase48, and several of its targets have been identified. For example, LCAD — a protein involved in fatty acid oxidation — is a prime target for SIRT3 deacetylation during prolonged fasting, resulting in the activation of fatty acid breakdown49. As a result, deletion of *Sirt3* impairs fat breakdown, exacerbating diet-induced obesity, and rendering these mice cold sensitive upon fasting50. The role for SIRT3 in fasting was further confirmed by the identification of multiple SIRT3 deacetylation sites in the enzyme 3-hydroxy-3-methylglutaryl CoA synthase 2, which regulates the production of ketone bodies, an important energy source for the brain when blood glucose levels are low51. An additional level of metabolic control by SIRT3 involves CR-induced deacetylation and activation of isocitrate dehydrogenase 2 (which is involved in the TCA

cycle)52, and deacetylation of components of complexes I53, II54 and III55 of oxidative phosphorylation, the final stage of mitochondrial aerobic respiration.

More recently, it became apparent that SIRT3 also affects oxidative stress defense, protecting the cell from reactive oxygen species (ROS) that are a by-product of oxidative phosphorylation52,55,56. Indeed, during caloric restriction, SIRT3 activates superoxide dismutase 2 (SOD2)56, a key mitochondrial antioxidant enzyme. Additionally, CR induces the SIRT3-mediated deacetylation of isocitrate dehydrogenase 2 in various tissues including inner ear cells, and thereby increases the ratio of reduced to oxidized glutathione, attenuating ROS levels52. As a result, CR protects against age-related hearing loss in a *Sirt3*-dependent fashion52.

SIRT3 also deacetylates and activates glutamate dehydrogenase (GDH)48,57, an enzyme controlling the TCA cycle, but the physiological relevance of this process is unclear. Finally, it is important to realize that most data regarding SIRT3 function are derived from the analysis of germline *Sirt3*$^{-/-}$ mice and studies in somatic *Sirt3*$^{-/-}$ mice will be necessary to address tissue contributions.

Sirtuin function — other sirtuins

Compared to SIRT1 and SIRT3, not much is known about the physiology of the other sirtuins. The cytosolic SIRT2 deacetylates tubulin58, but the relevance of this is unclear. More importantly, SIRT2 also deacetylates PAR-3, which in turn decreases the activity of the cell polarity control protein atypical protein kinase C (aPKC), thereby changing myelin formation of Schwann cells59. Additionally, in glucose-deprived conditions SIRT2 deacetylates phosphoenolpyruvate carboxykinase (PEPCK), which is involved in gluconeogenesis; this stabilizes the protein and prevents its ubiquitinylation-dependent degradation60. SIRT2 was also shown to deacetylate and activate FOXO1 impacting as such on adipogenesis61. The mitochondrial SIRT4, ADP-ribosylates GDH, inhibiting its activity and blocking amino acid-induced insulin secretion17. As a consequence, *Sirt4*$^{-/-}$ mice have increased plasma insulin levels, both in fed and fasted state, and when stimulated with glutamine17. SIRT4 also regulates fatty acid oxidation in hepatocytes and myocytes, and shRNA-mediated *Sirt4* knockdown in liver increased fatty acid oxidation62. It is interesting to note that SIRT3 and SIRT4 have apparently opposing roles in the regulation of GDH17,48,57 and fatty acid oxidation49,62, which will require further study to define how these two NAD^+-dependent enzymes integrate similar nutrient states into divergent responses.

The only target described for SIRT5 is CPS1, whose deacetylation during fasting activates ammonia detoxification through the urea cycle21. The primary function of SIRT5, however, may not act as a deacetylase21, but rather as a demalonylase and desuccinylase22, even of the described deacetylase target CPS123. Future studies will have to determine whether SIRT5 indeed has deacetylase activity in vivo, and whether these different posttranslational modifications—even on the same protein—indeed co-exist.

SIRT6 is involved in genomic DNA stability and repair, which plays a role in metabolism and aging. *Sirt6*$^{-/-}$ mice die early in life,14, have reduced IGF1 levels and are severely

hypoglycemic14, possibly mediated by hypoxia-inducible factor 1 (HIF1α)-dependent activation of glycolysis20. This switch towards glycolysis causes increased glucose uptake in muscle and brown adipose tissue, explaining the fatal hypoglycemia that these mice developed20. Interestingly, mice with neural-specific ablation of *Sirt6* are small at birth due to reduced growth hormone and IGF1 levels, reach normal body weight at 1 year of age, and become obese later in life, effects that are accompanied by strong hyperacetylation of histone H3K9 and H3K5663.

Finally, SIRT7 was reported to activate RNA polymerase I transcription, although its protein substrate is still unknown15. Another function for SIRT7 is derived from the study of *Sirt7*$^{-/-}$ mice, which display cardiac hypertrophy accompanying reduced lifespan64. The cardiac dysfunction is linked to p53 hyperacetylation64, but further studies will have to determine if p53 is indeed deacetylated by both SIRT1 and SIRT7, and if so, whether and how these two sirtuins interconnect on this target.

Regulation of sirtuin activity

Regulation of sirtuin activity occurs at various levels. As mentioned above, subcellular localization partly determines activity. Additional regulation is required, however, as several sirtuins share the same compartment — for example, SIRT3-SIRT5 — but also to allow specification of sirtuin activity towards distinct substrates. Transcriptional regulation, post-translational modifications, protein complex formation and enzymatic substrate levels all contribute to the activity of sirtuins to different degrees, even though not much is known about the extent of which they contribute to their regulation, especially in vivo. Nutrients and small molecules can also modulate sirtuin activity, opening opportunities for therapeutic interventions. We focus primarily on the best-described sirtuin, SIRT1, but discuss the regulation of other sirtuins where appropriate.

Regulation by expression

SIRT1 expression changes in various physiological conditions, resulting in induction during low energy status, and repression during energy excess states. For instance, nutrient starvation increases65, whereas high-fat diet reduces SIRT1 expression66. *SIRT1* promoter analysis revealed binding sites for various transcription factors (FOXO165, CREB (cAMP response element-binding) and ChREBP (carbohydrate response element-binding protein)) 67 and PPAR response elements68,69, which suggests that these transcription factors regulate SIRT1 expression in response to these stimuli. Indeed, FOXO165, PPARα68**,** PPARβ/δ70 and CREB67 increase SIRT1 levels, whereas PPARγ69 and ChREBP67 repress SIRT1 expression (FIG. 1). In addition, HIC1 (hypermethylated in cancer 1) functions as a transcriptional repressor of SIRT171. This repression is mediated by the transcriptional repressor CtBP (carboxyl-terminal binding protein), and is enhanced by NADH72 (FIG. 1), in line with repression of SIRT1 expression in times of energy excess. Finally, poly(ADP-ribose) polymerase 2 (PARP2), which belongs to a family of nuclear enzymes involved in DNA repair, apoptosis and transcription, binds and represses the *SIRT1* promoter (see below), although the exact mechanism is not yet understood73 (FIG. 1). Importantly, CREB,

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ChREBP67 and PARP273, not only regulate sirtuin expression *in vitro* but also have been shown to control its expression *in vivo*.

At a different level of regulation, microRNAs (miRNAs) modulate mRNA levels through the degradation of the primary transcript, or by inhibition of translation. As such, mouse miR-34a represses SIRT1 expression following genotoxic stress74. Interestingly, the miR-34a-mediated SIRT1 repression was increased in diet-induced obesity75, suggesting a physiological relevance for this interaction. miR-199a also represses SIRT1 expression (FIG. 1), as well as that of the oxygen sensor HIF1α76. During hypoxia, miR-199a is repressed in cardiac myocytes, allowing SIRT1 and HIF1α expression, thereby stabilizing p53 and reducing apoptosis76.

Although not much is known about the transcriptional control of the other sirtuins, gain-of-function studies revealed that SIRT3 expression is activated by ERRα, a nuclear receptor controlling mitochondrial function**,** which, together with PGC1α, binds the *Sirt3* promoter and controls the expression of genes involved in brown adipose tissue development and function77. The extent of *Sirt3* transcriptional regulation in physiological processes is not clear at present as illustrated by the fact that *Sirt3* mRNA expression increased after one week of high-fat diet but decreased after 13 weeks of diet50.

Regulation by post-translational modifications

Regulation of sirtuin activity by post-translational modifications is poorly understood. Several phosphorylation sites on SIRT1 have been identified78. SIRT1 is phosphorylated *in vitro* by cyclinB–Cdk1, which binds SIRT1, and mutation of the phosphorylation sites disturbs normal cell cycle progression78 (FIG. 1). The cJUN N-terminal kinase (JNK) also phosphorylates SIRT1 at three residues, particularly during oxidative stress79, and this results in deacetylation of histone H3, but not of p5379, suggesting that phosphorylation directs SIRT1 to specific targets (FIG. 1). In addition, the dual specificity tyrosine-phosphorylated and regulated kinases DYRK1 and DYRK3 phosphorylate SIRT1 at the Thr^{522} residue80. This activating phosphorylation leads to enhanced SIRT1-mediated p53 deacetylation and prevents apoptosis within the context of genotoxic stress80 (FIG. 1).

SIRT1 has also been shown to be sumoylated, which in cultured cells increases its activity81. Following genotoxic stress, for instance ultraviolet light or hydrogen peroxide, the desumoylase SENP removes this modification, inactivating SIRT1 and promoting cell death81 (FIG. 1). It is tempting to speculate that such conditions also activate the PARP enzymes — which constitute a family of major NAD^+ consumers — and thereby deplete NAD^+ levels, also inhibiting SIRT1 activity82. Whether these two events occur simultaneously would be interesting to explore.

Regulation by complex formation

Sirtuins are further regulated by forming complexes with other proteins. AROS (active regulator of SIRT1) is the only protein known to positively regulate SIRT1 following complex formation, thereby suppressing p5383.

By contrast, several negative regulators have been described. NCoR1 (nuclear receptor co-repressor 1) and SMRT (silencing mediator of retinoid and thyroid hormone receptors), form a complex with SIRT1 and PPARγ during fasting to repress the PPARγ-mediated induction of adipogenesis84.

DBC1 (deleted in breast cancer) binds the SIRT1 catalytic domain and inhibits its activity *in vitro* during genotoxic stress85,86. The physiological relevance of the DBC1-SIRT1 complex was confirmed, as complex formation was inhibited during fasting but increased in mice kept on a high-fat diet87. Consistent with this, deletion of *Dbc1* resulted in protection against high-fat diet-induced liver steatosis, even though these mice were — surprisingly — slightly heavier than control littermates87.

The histone methyltransferase LSD1 (lysine-specific demethylase 1) also interacts with the SIRT1 catalytic domain to regulate the expression of downstream Notch target genes88. SIRT1-mediated deacetylation of both H4K16 and H1K26, in conjunction with LSD1-mediated demethylation of H3K4, results in convergent repression of Notch target genes88. As such, the abnormal wing development of *Drosophila melanogaster* Notch mutants can be rescued by deletion of either the flies orthologues *Sir2* or *Lsd1*88.

Regulation through NAD+

As mentioned above, sirtuins depend on the cofactor NAD^+ for their activity, and therefore the availability of NAD^+ is another point of regulation.

NAD+ levels are modulated in several ways. For instance, a regulation of relative NAD^+ levels lies in the conversion to its reduced form NADH, for instance in glycolysis, thereby limiting the availability of NAD^+ to serve as a sirtuin substrate39. During fasting and exercise muscle NAD^+ levels rise, with a concomitant activation of sirtuins38. Similarly, caloric restriction increases NAD^+ levels in muscle, liver and white adipose tissue (WAT)89. By contrast, high-fat diet in mice reduces NAD^+/NADH ratio90.

NAD^+ can be synthesized from various precursors. De novo biosynthesis starts from the amino acid tryptophan and occurs primarily in the liver and kidney (reviewed in 24). However, NAD^+ can also be synthesized from nicotinic acid (known as the Preiss-Handler pathway) or nicotinamide (through the salvage pathway), both of which are present in our diet as vitamin B391 (FIG. 1). Interestingly, nicotinamide riboside, which is found in milk and already known to boost NAD^+ synthesis in bacteria, was recently demonstrated to act as a NAD^+ precursor and to enhance NAD^+ synthesis through the salvage pathway in eukaryotic cells92. Additionally, treatment of mice with another NAD^+ precursor — nicotinamide mononucleotide — activates SIRT1 and improves glucose tolerance in mice93, although it should be noted that this precursor does not occur in our diet. Future studies will have to determine whether the naturally occurring nicotinic acid, nicotinamide and/or nicotinamide riboside can activate sirtuins *in vivo*, and clarify the physiological importance of these precursors.

In addition to biosynthesis, manipulating the activities of NAD^+-depleting enzymes would also alter NAD^+ levels, thereby regulating sirtuin activity. PARPs are considered the major

NAD^+ degrading enzymes94,95. When activated by DNA damage they catalyze the transfer of ADP-ribose units from NAD^+ to substrate proteins to form branched polymers of ADP-ribose, leading to the decline of intracellular NAD^+ levels24.

As SIRT1 and PARPs compete for the same intracellular NAD^+ pool, a functional link between PARPs and SIRT1 has been proposed. Indeed, two recent reports revealed that the deletion of *Parp1* and *Parp2* in mice activates SIRT1, leading to increased numbers of mitochondria, enhanced energy expenditure and protection from diet-induced obesity73,82. *Parp1* and *Parp2* deletions activate SIRT1 via two distinct mechanisms: deletion of *Parp1* boosts the intracellular NAD^+ levels; and deletion of *Parp2*, which is a repressor of *SIRT1* expression (see above), increases *SIRT1* expression. Treatment of cells or mice with the pan-PARP inhibitor PJ34 results also in SIRT1 activation similar to that observed after *Parp* deletion82. Consistent with nuclear localization of both SIRT1 and PARPs, *Parp1* deletion does not increase the deacetylation activity of cytosolic SIRT2 or mitochondrial SIRT373,82, suggesting that the increase in NAD^+ is confined to the nuclear compartment

Similarly to PARP depletion, deletion of cADP-ribose synthase 38 (CD38), another important NAD^+ consumer, also increases NAD^+ levels, SIRT1 activity and mimics the metabolic phenotype expected for SIRT1 activation96. Interestingly, specific CD38 inhibitors have also been developed97, which together with PARP inhibitors, open new avenues for pharmacological sirtuin activation (FIG. 1). It will be important to determine whether these strategies are specific for SIRT1, or whether more sirtuins will be affected.

Compounds regulating sirtuin activity

Natural compounds that activate SIRT1 have been identified, providing further insights into sirtuin regulation (reviewed in 98). A small molecule screen in yeast revealed that several plant polyphenols — notably resveratrol — can induce SIRT1 to deacetylate p53-based peptides *in vitro* and treatment of *S. cerevisiae* with these compounds increased lifespan9, although some doubt surrounds these results (Box 2). Resveratrol also increases SIRT1 activity and enhances mitochondrial function in mice41,42, protecting them against diet-induced obesity and improving exercise performance and cold-resistance41. When fed a high-fat diet, resveratrol-treated mice also live longer42,99. Resveratrol improves mitochondrial activity and metabolic control in humans as well100. Interestingly, in humans significant lower resveratrol doses (~200-fold) resulted in similar plasma resveratrol levels, AMPK activation and comparable physiological effects compared to mice100. Several synthetic compounds have also been described that can activate the SIRT1 pathway. The most potent of these is SRT1720101, which, similarly to resveratrol, protects against diet-induced obesity by improving mitochondrial function40 and extends the lifespan of obese mice37.

Even though the physiological effects of resveratrol and SRT1720 are widely accepted, the mechanism by which they activate SIRT1 is unclear. Originally, these compounds were thought to directly activate SIRT1 by changing its enzymatic properties — lowering its K_m for both the protein substrate and NAD^+[9,101] — but more recent reports contest these results38,39,102–105. Instead of activating SIRT1, resveratrol was shown to activate AMPK40,42, possibly through inhibition of oxidative phosphorylation106 Arguing in favor

of the importance of AMPK is that the resveratrol effect was lost in AMPK-deficient mice38,104, even though one could argue that this does not demonstrate directionality of the AMPK-SIRT1 interaction. In these same mice, however, the NAD^+/NADH ratio increased by resveratrol in an AMPK-dependent manner, allowing downstream activation of sirtuins104 (FIG. 1). Similarly, established AMPK agonists, such as AICAR, increase NAD^+ levels and activate SIRT1-dependent PGC1α deacetylation39, and time-course experiments revealed that AMPK activation by exercise or fasting precedes a rise in NAD^+ levels and SIRT1 activation38.

Sirtuins in glucose metabolism

The maintenance of relatively constant blood glucose concentrations is essential to provide energy to tissues, most importantly for an uninterrupted glucose supply to the brain, which almost exclusively uses glucose as an energy source. Glucose levels are regulated through various processes, including intestinal glucose uptake, hepatic glucose output, and glucose uptake, utilization and storage in peripheral tissues. The main regulating hormone — insulin — promotes glucose uptake in peripheral tissues (muscle and WAT), glycolysis and storage of glucose as glycogen in the fed state. Glucagon counteracts the effect of insulin and stimulates hepatic glucose production during fasting. Sirtuins modulate a range of cellular processes involved in maintaining glucose homeostasis in tissues such as muscle, WAT, liver and pancreas (FIG. 4).

Gluconeogenesis and insulin sensitivity

Gluconeogenesis is a cytosolic process that increases glucose production, mainly from the liver, in situations when the supply of energy is limiting such as during fasting, calorie restriction and exercise107. Intriguingly, SIRT1 has a dual and controversial role in the control of gluconeogenesis. On the one hand, SIRT1 can suppress hepatic glucose production by deacetylating the transcription factor CRTC2 (CREB-regulated transcription coactivator 2), leading to CRTC2 degradation and consequent reduction in the transcription of gluconeogenic genes (FIG. 2 and 4)108. On the other hand, SIRT1 can also stimulate the gluconeogenic transcriptional program by deacetylating and activating FOXO1108,109 and PGC1α36 (FIG. 2 and 4). However, it should be noted that genetically increased PGC1α expression clearly enhances gluconeogenesis but the evidence supporting the relevance of physiological modulation of PGC1α in the control of gluconeogenesis is weak110. Given that the exact contribution of the activities of CRTC2, FOXO1 and PGC1α to gluconeogenesis is still under debate, it is unclear which of these above-mentioned SIRT1 actions is the primary and/or predominant event. Furthermore, recent studies revealing that a temporal regulation of SIRT1 expression and deacetylation of SIRT1's targets such as CRTC2 is central to the control of the appropriate rate of gluconeogenesis67,108 have made this story even more intricate.

In contrast to SIRT1, SIRT2-4 are thought to maintain gluconeogenesis, in particular during times of energy limitation. Specifically, SIRT2 deacetylates and increases the stability of the gluconeogenic enzyme PEPCK upon glucose deprivation60 (FIG. 2). Furthermore, SIRT3 and SIRT4 may regulate gluconeogenesis from amino acids during caloric restriction via

their target GDH, a mitochondrial enzyme converting glutamate to α-ketoglutarate, facilitating glucose production via TCA cycle 17,48,57 (FIG. 2 and 4). To fully understand the role of these sirtuins in the regulation of gluconeogenesis, further studies are clearly needed.

Despite the conflicting findings in gluconeogenesis, it is tempting to propose that SIRT1 may function as an insulin sensitizer on the basis of the metabolic changes (lowered fasting glucose levels and/or hepatic glucose production) observed in SIRT1 transgenic mice111, after adenovirus-mediated SIRT1 overexpression108, in SIRT1 activator-treated mice40,42,101, and in resveratrol-treated obese humans100. Moreover, SIRT1 activation also protects from diet-induced and genetic insulin resistance in mice42,100,101, and *SIRT1* mRNA expression in adipose tissue positively correlates with insulin sensitivity in humans during hyperinsulinaemia112. Mice lacking SIRT1 specifically in the liver have been shown to develop insulin resistance113 or maintain normal glucose homeostasis89,114, whereas acute adenoviral liver SIRT1 knockdown induces fasting hypoglycaemia115. This suggests that the effect of acute SIRT1 liver knockdown on glucose metabolism may be compensated in situations of chronic SIRT1 deficiency. Furthermore, SIRT3 also plays a role in insulin sensitization as its absence may contribute to development of insulin resistance in the muscle via increasing ROS production and impairing mitochondrial oxidation55. Based on these studies, the pharmacological targeting of SIRT1 is a promising treatment for type 2 diabetes.

Glycolysis

Glycolysis is the main pathway for glucose utilization in the fed state, in which glucose is catabolized into pyruvate in the cytosol107. Under anaerobic conditions (e.g. in exercising muscle), pyruvate is converted by lactate dehydrogenase into lactate. In aerobic conditions, energy from glucose is released through glucose oxidation, a process in which pyruvate and the reducing equivalents produced during glycolysis fuel mitochondrial oxidative phosphorylation to generate ATP.

Recent studies have provided insights into the role of different sirtuins in glycolysis. It is now well established that SIRT1 is involved in the control of glycolysis by activating PGC1α, which attenuates the transcription of glycolytic genes36 (FIG. 2 and 4). Furthermore, SIRT1, SIRT3 and SIRT6 all suppress the transcription factor HIF1α, which represses glucose oxidation through TCA cycle116. SIRT1 suppresses HIF1α directly through deacetylation117 (FIG. 2 and 4), whereas SIRT3 inhibits the ROS-mediated stabilization of HIF1α by activating SOD2118 and possibly increasing reduced glutathione levels52 and thereby enhancing cellular antioxidant defenses (FIG. 2 and 4). SIRT6 acts as a corepressor of HIF1α to diminish glycolysis during the normal nutritional state20 (FIG. 2 and 4).

When integrating these observations, SIRT1, SIRT3 and SIRT6 act to inhibit glycolysis and stimulate mitochondrial oxidation of fatty acids. This finding is particularly interesting, as tumor cells have a high reliance on glycolysis, known as the Warburg effect. Therefore, this switch from glycolysis to oxidative metabolism induced by sirtuin activators117,118 and PARP inhibitors82, which indirectly activate SIRT1, could contribute to their anti-tumor activity.

Insulin secretion

The secretion of the hormone insulin from pancreatic β-cells is tightly coupled to blood glucose levels, via an intricate signalling pathway119. After entering the *β*-cell via glucose transporter 2, glucose is metabolized to generate ATP through glucose oxidation. The subsequent increase in ATP/ADP ratio closes ATP-sensitive potassium channels, leading to *β*-cell depolarization and calcium influx, ultimately coupling insulin release to plasma glucose levels. Although glucose is the most potent stimulator of insulin secretion it is also induced by some amino acids.

SIRT1 has been shown to induce glucose-stimulated insulin secretion by increasing the yield of ATP produced from glucose oxidation. This is achieved through transcriptional repression of the uncoupling protein 2 (UCP2) (FIG. 2 and 4), which uncouples mitochondrial ATP production during oxidative phosphorylation. As such, SIRT1 deficiency results in high UCP2 levels and diminished insulin secretion120,121.

As discussed above, both SIRT3 and SIRT4 target GDH (FIG. 2 and 4), although in opposite fashion, and thereby impact on amino-acid induced insulin secretion during calorie restriction as GDH catalyzes formation of ∝-ketoglutarate, which can be used for ATP production via the TCA cycle and oxidative phosphorylation. Specifically, SIRT4 seems to inhibit insulin secretion, as SIRT4 deficiency increases GDH activity in pancreatic islet cells and increases amino acid-stimulated insulin secretion17. Furthermore, SIRT4 overexpression in insulinoma cells leads to blunted insulin secretion122. Although SIRT3 has been proposed to activate GDH48,57, GDH activity is unchanged in *Sirt3*$^{-/-}$ mice21, so the physiological role of SIRT3 in GDH regulation and insulin secretion is unclear.

SIRT1 and SIRT4 therefore seem to control insulin secretion in opposing directions in response to feeding and calorie restriction. Although the elucidation of underlying mechanisms is under way, the current findings suggest the existence of a sirtuin network to maintain proper insulin secretion.

Sirtuins in lipid metabolism

Lipid metabolism comprises lipid synthesis, uptake, storage and utilization, which requires tight control, for example during periods of fasting or prolonged exercise. Insulin promotes hepatic triglyceride synthesis and storage of triglycerides in WAT upon feeding, whereas glucagon and epinephrin stimulates lipolysis in the WAT and fatty acid oxidation in other tissues when nutrients are limiting. Sirtuins influence diverse aspects of lipid homeostasis in multiple tissues; here we concentrate on their effects in WAT, liver and skeletal muscle (FIG. 4). We do not discuss the role of sirtuins in cholesterol and bile acid metabolism (for a review, see 123).

Lipid synthesis

When whole body energy stores are maximal, excess glucose, fatty acids and amino acids are used in the liver to synthesize fatty acids, which are exported to WAT where they are stored as triacylglycerols124. Fatty acid synthesis occurs in cytosol where acetyl-CoA, derived from fuel catabolism, and malonyl-CoA are used as substrates for fatty acids

production, catalyzed by fatty acid synthase. A key transcription factor controlling the expression of genes involved in lipid synthesis is liver X receptor (LXR), which in part mediates its effect through the induction of the sterol-response element-binding protein 1c (SREBP1c)125. SIRT1 deacetylates and subsequently increases the transcriptional activity of LXR126. Therefore, SIRT1 activation could theoretically increase fatty acid synthesis. However, SIRT1 also deacetylates the downstream target of LXR, SREBP1c, thereby destabilizing it and reducing its occupancy on the lipogenic gene promoters leading to suppression of fatty acid synthesis (FIG. 3 and 4)127,128. This notion is supported by the fact that *Sirt1* transgenic mice are protected from hepatic steatosis129 and *Sirt1*$^{-/-}$ mice are prone to develop hepatic steatosis114,130 (see Lipid utilization and energy expenditure section). Therefore, SIRT1-mediated LXR activation seems to target cholesterol metabolism (for a review, see 123) rather than triglyceride synthesis.

SIRT6 has also been implicated in the control of fatty acid synthesis (FIG. 3). Indeed, genes involved in fatty acid transport and lipogenesis are induced in liver-specific *Sirt6*$^{-/-}$ mice131, suggesting that SIRT6 serves as a negative regulator of triglyceride synthesis.

Lipid storage

Fatty acids can be stored as cytosolic triacylglycerols droplets in all cells, but the prime storage occurs in adipocytes of WAT132. The development of adipocytes from preadipocytes — adipogenesis — is controlled by a complex transcriptional program coordinated mainly by the nuclear receptor PPARγ, the master regulator of adipocyte differentiation133.

In differentiated adipocyte cell lines, SIRT1 inhibits adipogenesis and enhances fat mobilization through lipolysis84 by suppressing the activity of PPARγ. SIRT1 achieves this by promoting the assembly of a corepressor complex, involving NCoR1 and SMRT, on the promoters of PPARγ target genes to repress their transcription (FIG. 3 and 4) and limit fat storage in situations of caloric restriction and fasting84. Cell-based studies have suggested that SIRT2 may also inhibit adipogenesis and promote lipolysis during nutrient deprivation, by deacetylating and activating FOXO1 (FIG. 3), promoting the binding of FOXO1 to PPARγ and repressing its transcriptional activity61,134. However, further research is required to establish the existence of a mechanistic link between SIRT1 and SIRT2 with PPARγ and FOXO1 in vivo.

Lipid utilization and energy expenditure

Fatty acid oxidation occurs mainly in the mitochondrial matrix. Therefore, long-chain fatty acids need first to be transported from the cytoplasm into mitochondria135, where they undergo β-oxidation to generate acetyl-CoAs, which can be used for ATP production via TCA cycle and oxidative phosphorylation. As malonyl-CoA, an intermediate of fatty acid synthesis, inhibits carnitine palmitoyltransferase, the enzyme controlling the mitochondrial import of fatty acids, low levels of malonyl-CoA facilitate oxidation of fatty acids.

Fatty acid oxidation is a major determinant of whole body energy expenditure as it consumes high amounts of oxygen. SIRT1 enhances energy expenditure by stimulating fatty acid oxidation, as well as oxidative phosphorylation, in response to fasting. This is achieved through activating PPARα and its coactivator PGC1α114 that stimulate expression of genes

involved in fatty acid uptake and/or β-oxidation (FIG. 3 and 4). In support of this notion, SIRT1 activation protects mice from diet-induced obesity by increasing the rate of fatty acid oxidation 40,41. Furthermore, in humans, *SIRT1* mRNA expression positively correlates with energy expenditure during hyperinsulinaemic clamp112, the gold standard to measure insulin sensitivity, and three *Sirt1* single-nucleotide polymorphisms are associated with whole body energy expenditure in Finnish subjects41, confirming the important and conserved role of SIRT1 in the modulation of whole body energy expenditure.

Fatty acids are an important source of energy in the liver during fasting, CR and high-fat feeding. SIRT1 has been shown to be an important regulator of fatty acid oxidation in the liver. Indeed, induction of fatty acid oxidation through activation of PPARα and PGC1α and their target genes is impaired during fasting in mice lacking SIRT1 specifically in the liver114,115. As reduced fatty acid oxidation correlates with hepatic steatosis, liver-specific and heterozygous germline *Sirt1*$^{-/-}$ mice are susceptible to hepatic steatosis under standard and high-fat diets114,130,136. Surprisingly, one strain of liver-specific *Sirt1*$^{-/-}$ mice is protected from hepatic steatosis and diet-induced obesity89. Despite this, most findings support that SIRT1 stimulates lipid utilization. For example adenoviral overexpression of SIRT1137, *Sirt1* transgenic mice129, and pharmacological SIRT1 activation40,127,128,138,139 improve liver steatosis in diet-induced and genetically obese mouse models. In addition, resveratrol treatment reduces hepatic fat content in obese humans100.

Of the other sirtuins, SIRT3, SIRT4 and SIRT6 are also involved in the regulation of fatty acid oxidation in the liver. As discussed above, SIRT3 deacetylates and activates LCAD49 (FIG. 2 and 3), a key enzyme involved in the oxidation of long-chain fatty acids. By contrast, SIRT4 seems to be a negative regulator of fatty acid oxidation62 (FIG. 3), whereas SIRT6 was reported to stimulate expression of genes involved in fatty acid oxidation as well as fatty acid oxidation 131, although the exact mechanism is unclear. Importantly, both SIRT3-deficient mice and liver-specific *Sirt6*$^{-/-}$ mice are more susceptible to hepatic steatosis49,131. Altogether, SIRT1, SIRT3 and SIRT6 may be potential therapeutic targets to treat liver diseases characterized by lipid accumulation.

Skeletal muscle uses fatty acids as a fuel during fasting, prolonged exercise and high-fat feeding. The switch to oxidative metabolism includes the induction of mitochondrial biogenesis, fatty acid oxidation and oxidative phosphorylation, processes that are controlled by the yin and yang between corepressors such as NCoR1140 and coactivators, such as PGC1α141,142, which determine the transcriptional activity of downstream targets controlling oxidative metabolism. However, what are the key events leading to the shift towards more oxidative metabolism in skeletal muscle? AMPK is a cellular energy sensor that is activated during exercise or energy demand by an increase in the AMP/ATP ratio[2] (FIG. 4). Activated AMPK phosphorylates PGC1α directly143 and also stimulates SIRT1 indirectly by boosting cellular NAD^+ levels39 (FIG. 4). Nevertheless, the activation of SIRT1 may be AMPK-independent in skeletal muscle during calorie restriction144,145 but this is not a consistent finding146. Once SIRT1 is activated by NAD^+, it deacetylates and locks PGC1α in an active state36, which together with reduced activity of NCoR1140 will favor oxidative metabolism (FIG. 4). Recent data have implied that the nuclear abundance of

the key PGC1α acetyltransferase, GCN5 (general control of amino-acid synthesis 5)35 also diminished in response to exercise, which could also contribute to the decreased PGC1α acetylation147. However, further work is needed to determine the function and regulation of coactivators, such as GCN535 and corepressors, such as NCoR1 in the control of oxidative metabolism. In addition to the role of SIRT1, SIRT3-dependent deacetylation and activation of LCAD49 and oxidative phosphorylation complexes53–55, as discussed above, might affect fatty acid oxidation in skeletal muscle.

Interestingly, in states of energy excess, for instance following high-fat feeding, these processes typifying situations of high energy demand, are reversed. As such, the cellular AMPK and SIRT1 activities are attenuated due to high intracellular ATP and low NAD^+ levels148 (FIG. 4). Furthermore, high-fat feeding induces the expression of GCN5 while concomitantly reducing SIRT1 levels, resulting in the inhibition of PGC1α by increasing its acetylation level66 (FIG. 4). Lastly, during energy excess NCoR1 is activated leading to decreased transcription of genes governing mitochondrial activity140.

Conclusions and future perspectives

Sirtuins constitute a protein family of metabolic sensors, translating changes in NAD^+ levels into adaptive responses. Although the relevance of SIRT1 as a *strictu sensu* longevity gene has been disputed (Box 2), it is evident that by regulating the activity of its target enzymes and transcription factors, sirtuins affect health in a pleiotropic manner. The fact that sirtuin activation prevents diet-induced obesity, and their overexpression prevents cancer risk, suggests that sirtuins are more involved in stress responses. This implies that while unstressed by for instance genotoxic or metabolic (high fat diet) stress, or ageing, activation or ectopic expression of sirtuins does not impact physiological fitness. On the other hand, following such external stressors, enhanced sirtuin activity serves to activate protective pathways, and can thereby impact lifespan. As such, they should still be considered as candidate targets for the prevention and/or treatment of age-related diseases and to increase healthspan. In fact, in contrast to increasing lifespan, which has limited medical relevance, improving healthspan has an immediate clinical and public health impact, given the ever increasing ‘greying’ of the world population.

Besides the relevance of sirtuin activators within the context of common diseases of ageing, it would be worth testing sirtuin activators for the treatment of more rare, but also more insidious, inherited diseases relating to mitochondrial metabolism. This was recently exemplified by treating fibroblasts of patients with a mitochondrial fatty acid oxidation disorder with resveratrol, which resulted in restoration of fatty acid degradation158. Altogether, although sirtuins might have lost their immaculate Methuselah image, they are still likely to help those in need for a metabolic Samaritan.

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Glossary terms

caloric restriction
A reduction of caloric intake (typically 20-50% less than average) that was shown to increase lifespan in a variety of organisms

ADP-ribosyltransferases
Enzymes that transfer an ADP-ribose group on a protein target

Demalonylase
Enzyme that removes a malonyl group from a specific lysine residue of a target protein.

Desuccinylate
Enzyme that removes a succinyl group from a specific lysine residue of a target protein.

Ketone body
Metabolic energy unit, derived from fat breakdown, which serves especially to fuel the brain in times of low blood glucose concentrations.

TCA cycle
Also known as Krebs cycle. Acetyl-CoA derived from glucose or fat breakdown is further metabolized to offer reduced energy equivalents that are used by mitochondrial oxidative phosphorylation to generate ATP.

Reactive oxygen species
Oxygen radicals that are produced by the mitochondrial respiratory chain. In excess, they can cause intracellular and mitochondrial damage, which promotes cell death.

K_m
Michaelis constant. Enzyme property describing the enzyme's substrate concentration at which the enzyme works at half-maximal capacity.

Oxidative phosphorylation
Mitochondrial enzymatic chain of events by which ATP is generated

Hyperinsulinaemia
A condition that occurs when there is excess of insulin in the circulation.

Fasting hypoglycaemia
A condition during fasting that occurs when circulating glucose levels are abnormally low.

Insulinoma cells
Tumor cells derived from pancreatic beta-cells which secrete insulin

Single-nucleotide polymorphisms

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A naturally occurring single nucleotide variation in a DNA sequence. The most common type of genetic polymorphism

Steatosis

A condition characterized by abnormal accumulation of lipid within the cell.

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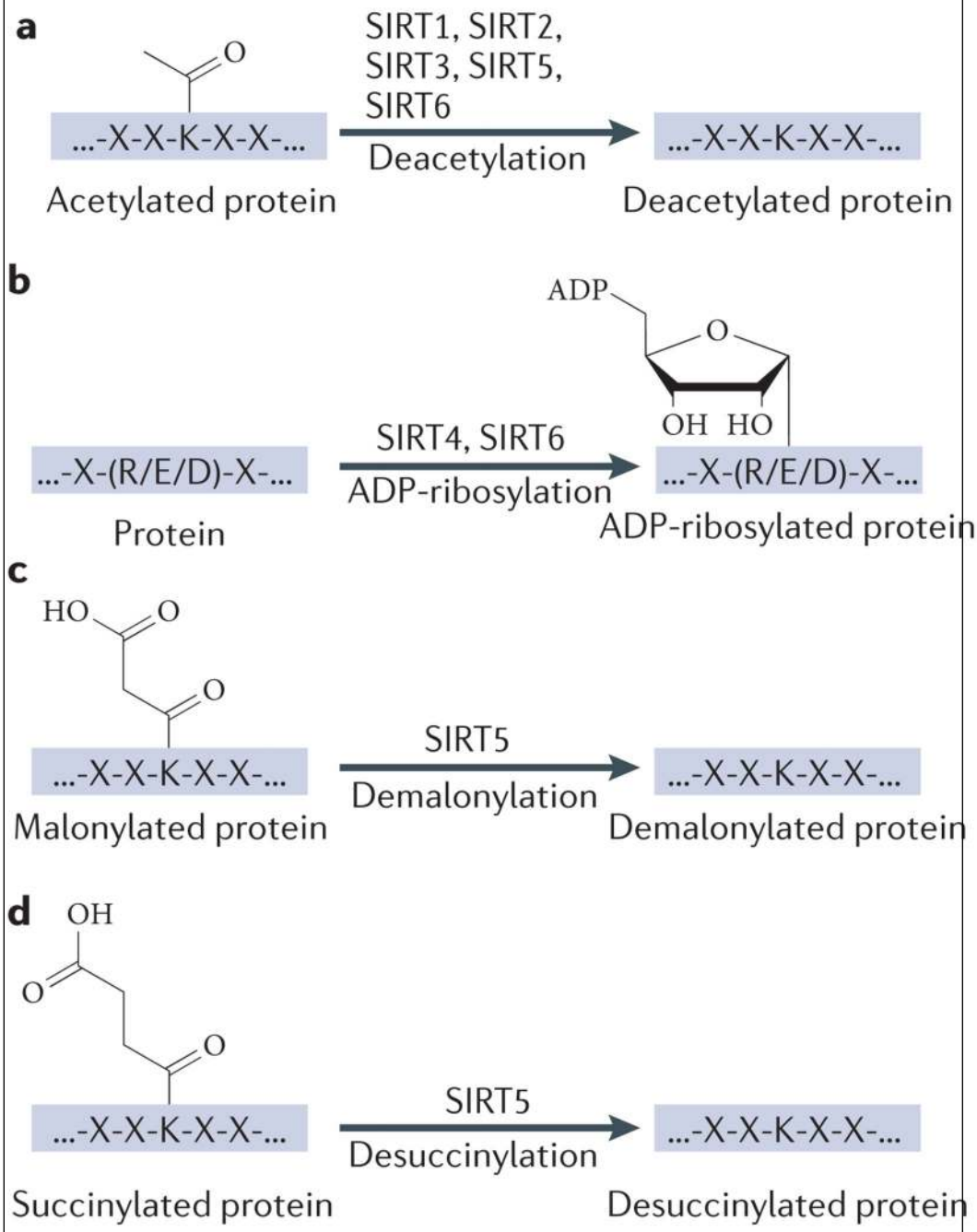
BOX1

Enzymatic activity of sirtuins



Although sirtuins were originally described as histone deacetylases (part **a,** removing an acetyl moiety from a histone/protein), a shift is taking place in how the field approaches the sirtuin enzymatic activity. Not only did the range of deacetylase targets broaden beyond histones, to include many transcription factors and enzymes (reviewed in 5,24,30), but also SIRT4 and SIRT6 were described to act as ADP-ribosyl

transferases17,18 (part **b,** transferring an ADP-ribose moiety onto a protein). Two recent papers22,23 now describe SIRT5 to possess demalonylation (part **c**, removing a malonyl moiety from a protein) and desuccinylation (part **d**, removing an succinyl moiety from a protein) activity. These new functions expose the possibility that sirtuins might in fact function as protein deacylases (removing any acyl moiety from a protein), with a wide variety of target proteins. The acetyl, malonyl, or succinyl moiety that is removed during such deacylase reaction, could also be considered ADP-ribosylated during this process23. It is tempting to speculate that the actual function of sirtuin is not to deacetylate proteins, but rather to function as ADP-ribosyl tranferase or deacylase, using either an unmodified protein as a target — e.g. ADP-ribosylation activity of SIRT4 and SIRT6 — or a protein modified with either an acetyl-, malonyl-, or succinyl moiety. This would in fact describe a more general activity that is true for all sirtuins, without discrimination of the actual target.

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BOX 2

SIRT1 and longevity – a shift from life-span towards health-span

The yeast Sir2 was described as a longevity protein7, and with time also worm *sir-2.1*149 and fly Sir2150 were implicated in lifespan regulation and thought to mediate the beneficial effects of caloric restriction on lifespan3. This role of sirtuins was seemingly corroborated by the beneficial effects of the sirtuin-activating compound resveratrol on yeast lifespan9, but other studies in *Drosophila melanogaster* and *Caenorhabditis elegans* have cast a shadow of doubt on the possibility that sirtuins mediate the effects of resveratrol and caloric restriction on longevity151,152. Furthermore, recent work demonstrates that the effect of overexpression of worm *sir-2.1* or fly *Sir2* on longevity is limited at best153,154.

Evidence for a role of SIRT1 in longevity is not clear in mammals either. Arguing for a role of *Sirt1* in CR-mediated longevity, *Sirt1*$^{-/-}$ mice do not show lifespan extension on a CR regimen155. SIRT1 transgenic mice display reduced cancer risk and are protected against metabolic dysfunction associated with aging, but do not show increased lifespan156. Mice treated with resveratrol99 or the synthetic sirtuin activator SRT172037 show increased lifespan, but only when metabolically stressed with a high-fat diet. However, it should be mentioned that there is concern that both resveratrol and SRT1720 might not target SIRT1 specifically102. In addition, no genetic association was found between polymorphisms in *Sirt1* and human lifespan157. Altogether, we hypothesize that SIRT1 activity may not determine natural lifespan, but rather plays a role in health maintenance and stress response, and upon activation extends lifespan or rescues stress-induced reductions in lifespan. Interestingly, SIRT6 is a strong determinant of lifespan, as *Sirt6*$^{-/-}$ mice show signs of accelerated aging14. Further studies in animal models and humans are needed to explore the effects of the other sirtuin family members on lifespan.

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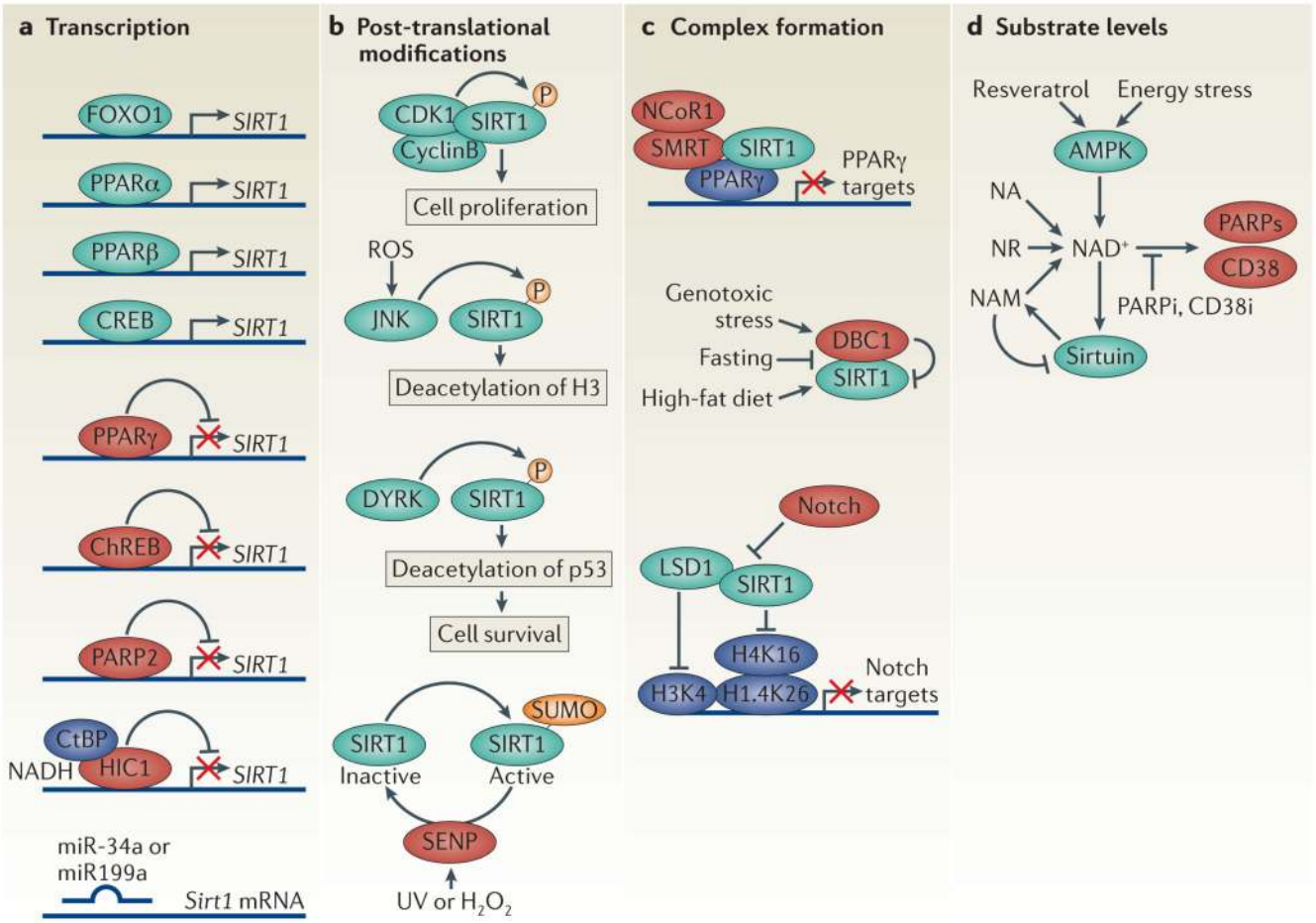

Figure 1. Regulation of sirtuin expression and activity.

a | Various transcription factors regulate sirtuin expression. FOXO1, PPARα, PPARβ/δ and CREB enhance SIRT1 (in green), whereas PPARγ, ChREBP, PARP2 and HIC1 repress SIRT1 expression (in red). Only CREB, ChREBP and PARP2 were also shown to possess this activity *in vivo*. *Sirt1* expression is also repressed by the miRNAs miR-34a and miR-199a. **b** | Reversible post-translational modifications affect SIRT1 activity. The cyclinB/Cdk1 complex phosphorylates (P) SIRT1 thereby allowing cell cycle progression. Activation of JNK by reactive oxygen species (ROS) results in SIRT1 phosphorylation and subsequent deacetylation of histone H3, but not p53. Genotoxic stress, for instance by UV light or H2O2 exposure, results in desumoylation of SIRT1, inactiving it. Sumoylation (sumo) by a yet unidentified enzyme activates SIRT1. **c** | Complex formation with other proteins influences SIRT1 enzymatic activity. A complex of NCoR1 and SIRT1 blocks the transcriptional activity of PPARγ. Genotoxic and metabolic (high-fat diet) stress induce DBC1-SIRT1 complex formation, by which DBC1 inactivates SIRT1. Fasting relieves this inactivation. The LSD1-SIRT1 complex represses Notch target gene expression by demethylation and deacetylation of specific histones, but is derepressed by activation of the Notch pathway. Complex formation with AROS activates SIRT1. **d** | Controlling the levels of the cofactor NAD^+ governs SIRT1 function. NAD^+ levels are increased by providing precursors nicotinic

acid (NA), nicotinamide (NAM) or nicotinamide riboside (NR), inhibiting its breakdown by means of PARP or CD38 inhibition, or by AMPK activation following energy stress or treatment with the AMPK activator resveratrol. Increased NAD^+ levels subsequently lead to sirtuin activation.

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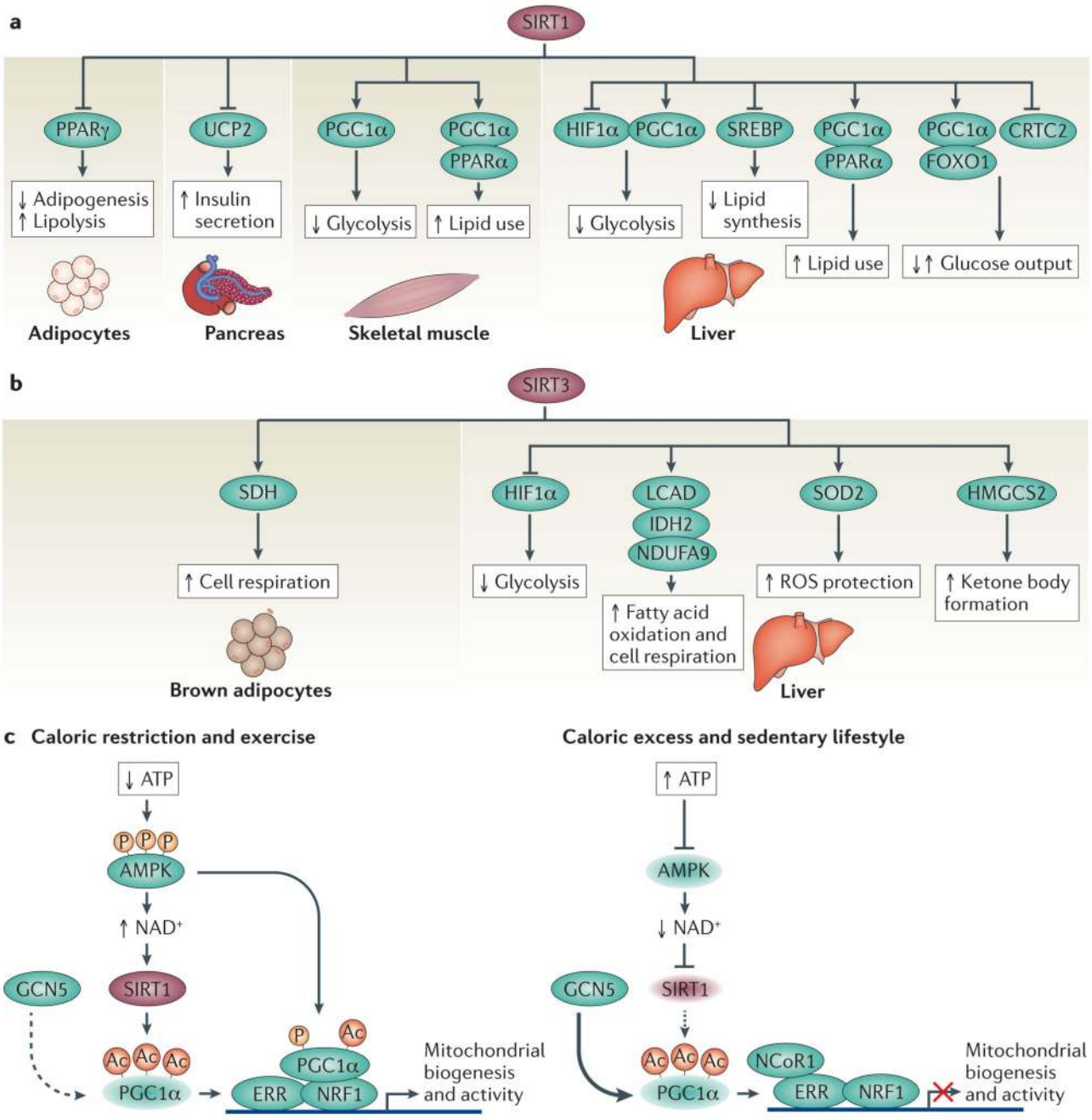

Figure 2. Overview of sirtuins' role in the regulation of pathways involved in glucose metabolism.

Red arrows indicate activating effects whereas blue bars denote inhibiting functions. Colored symbols show metabolic processes, genes or proteins, which are affected by the nuclear function(s) of SIRT1 or SIRT6. The involvement of certain specific sirtuins in the control of particular metabolic pathway is indicated with white oval.

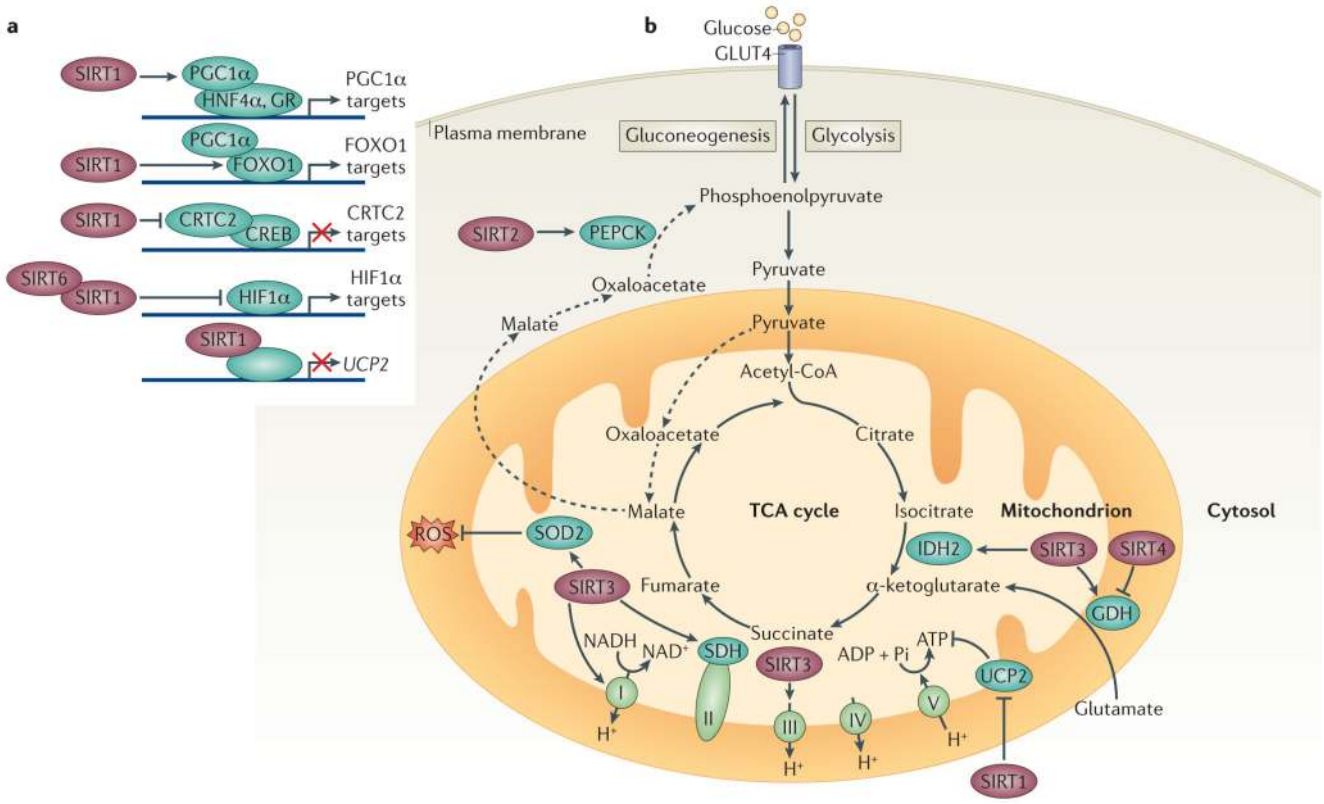

Figure 3. Overview of sirtuins' role in the regulation of lipid metabolism.

Red arrows indicate activating effects whereas blue bars denote inhibiting functions. Colored symbols show metabolic processes, genes or proteins, which are affected by the nuclear function(s) of SIRT1 or SIRT6. The involvement of certain specific sirtuins in the control of particular metabolic pathway is indicated with white oval.

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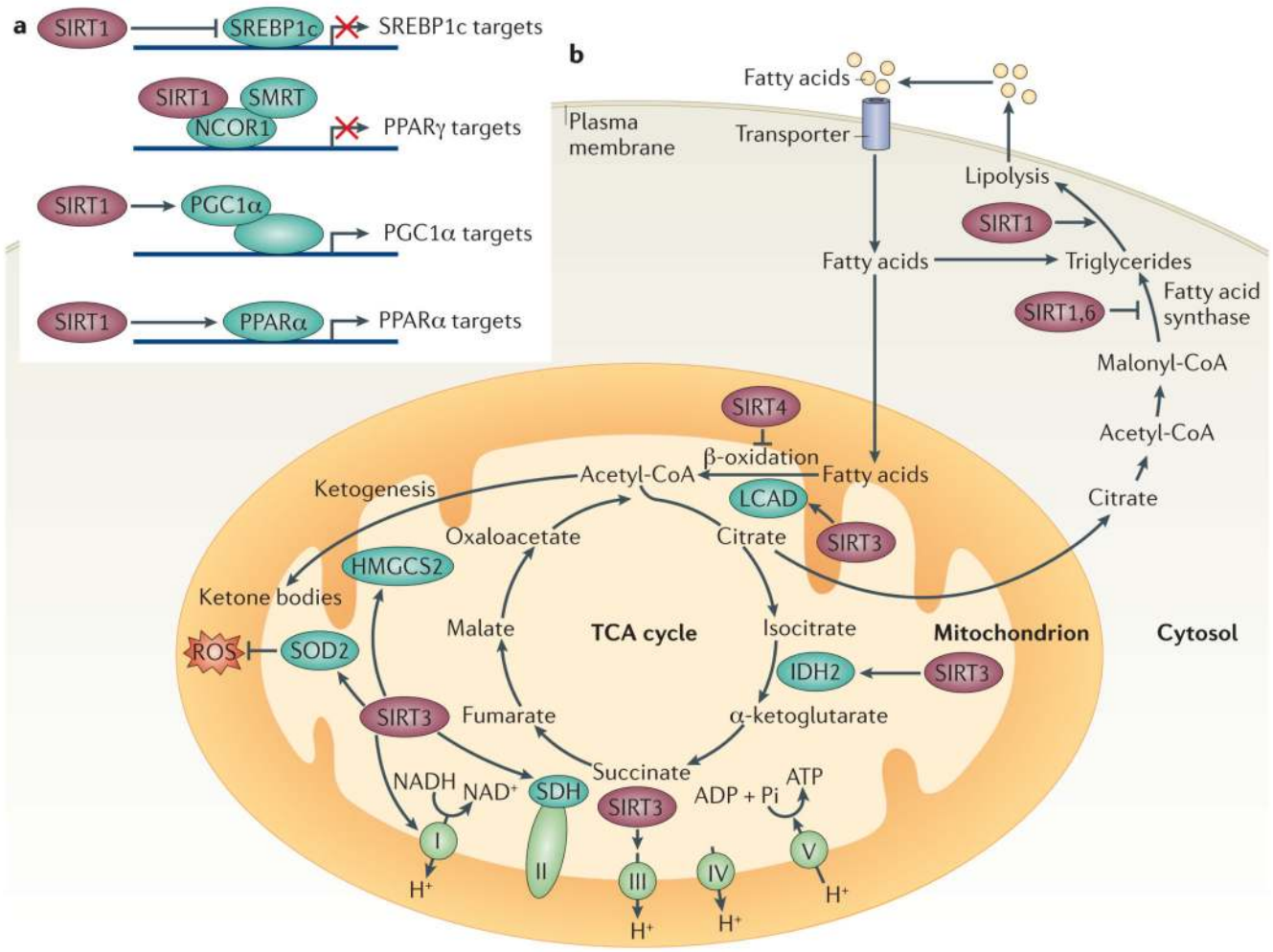

Figure 4. The association of sirtuin activity with metabolic health.

a | Sirtuins influence glucose and lipid metabolism in several tissues. SIRT1 inhibits adipogenesis in 3T3L1 adipocytes. In addition, SIRT1 decreases fat storage by enhances lipolysis through PPARγ in WAT. In pancreas, SIRT1 increases insulin secretion by repressing the transcription of UCP2. In skeletal muscle, SIRT1 attenuates glycolysis via PGC1α and enhances lipid utilization by stimulating PGC1α and PPARα. In the liver, SIRT1 decreases glycolysis via HIF1α and PGC1α and lowers lipid accumulation by suppressing lipid synthesis via SREBP-1c and promoting lipid utilization through PGC1α and PPARα. In addition, SIRT1 regulates hepatic glucose production via PGC1α, CRTC2 and FOXO1, although its exact role in this process is under debate. Using SIRT3 whole-body knock-out mice, it has been demonstrated that during energy limitation, SIRT3 suppresses glycolysis via HIF1α, promotes fatty acid oxidation through LCAD, IDH2 and NDUFA9, protects from ROS by stimulating SOD2 and increases ketone body formation through HMGCS2. Based on in vitro studies, SIRT3 promotes cellular respiration in brown adipocytes via SDH.

b | In response to calorie restriction and exercise, the induction of SIRT1 stimulates mitochondrial activity, leading to improved metabolism and disease prevention. During

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energy limitation, low ATP levels activate AMPK, which in turn induces SIRT1 by increasing NAD^+ levels. SIRT1 increases mitochondrial activity by decreasing acetylation levels of PGC1α. During calorie excess and sedentary life-style, cellular ATP levels increase, whereas NAD^+ levels decrease, thereby inhibiting SIRT1. As a result, PGC1α remains acetylated, which leads to decreased mitochondrial activity, predisposing to the development of metabolic diseases.

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Table 1

Sirtuin localization and function.

Sirtuin	Class	Localization	Activity	Targets	References
SIRT1	I	Nuclear, cytosolic	Deacetylation	PGC1α, FOXO1, FOXO3, p53, NOTCH, NF-κB, HIF1α, LXR, FXR, SREBP-1c, and more	5,30,32,33,39,41,42
SIRT2	I	Cytosolic	Deacetylation	Tubulin, PEPCK, FOXO1, PAR-3	58–61
SIRT3	I	Mitochondrial	Deacetylation	LCAD, HMGCS2, SOD2, GDH, IDH2, OXPHOS complexes, and more	46–49,51–57
SIRT4	II	Mitochondrial	ADP-ribosylation	GDH	17
SIRT5	III	Mitochondrial	Deacetylation, demalonylation, desuccinylation	CPS1	21–23
SIRT6	IV	Nuclear	Deacetylation, ADP-ribosylation	H3K9, H3K56	14,18–20,63
SIRT7	IV	Nucleolus	Unknown	unknown	15,64