Advances in Biochemistry & Applications in Medicine

Chapter 12

Sirtuins: Its Role in Metabolic Homeostasis

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1. Sirtuins: Its Role in Metabolic Homeostasis

Sirtuin proteins are evolutionarily conserved enzymes that function in critical cellular processes such as DNA repair, transcription and stress resistance. The name Sirtuin (Sir2) is derived from the yeast gene 'silent information regulator' which is responsible for controlled expression of the silent mating type loci and also required for telomere hyper cluster formation in quiescent yeast cells [1]. It was not until fifteen years later the significance of sir2 proteins was identified as beta-nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases, which deacetylate lysine at a specific site and accounts for silencing, recombination suppression, apoptosis, mitochondrial biogenesis, lipid metabolism, and extension of life span *in-vivo* [2,3]. The sirtuins were originally classified as histone deacetylases but many non-histone targets are described recently.

1.1. Biochemistry of sirtuins

Sirtuins possess a conserved catalytic core (~275 amino acids) that is flanked by N- and C- terminal extensions. These N- and C- terminal extensions play a key role in ensuring proper cellular localization, regulating the interaction with other proteins and targets for post-translational modifications that affect the functions of sirtuins [4]. The larger sirtuin domain consists of a Rossmann-fold, that is characteristic for NAD^+-binding unit, and the smaller domain: a

zinc-binding motif and a α-helical region which shows the highest diversity among family members [5].

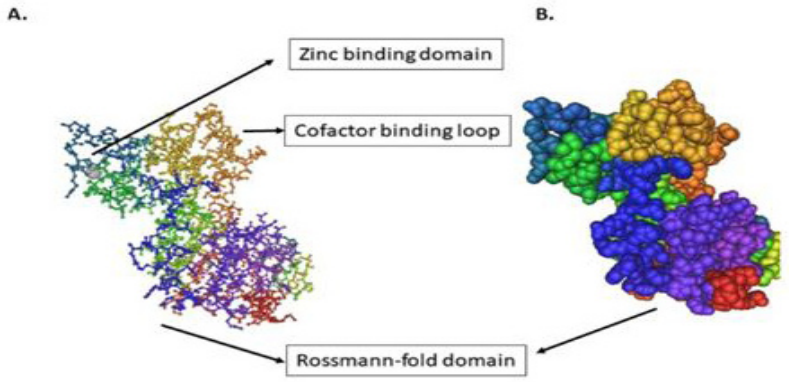

Figure 1: structure of NAD-dependent protien Deacetylase Sirtuin-1 (PDB ID 41G9): A. Ball and stick model B. space fill model

Protein acetylation is regulated by protein acetyltransferases and deacetylases. Sirtuins are a family of NAD^+ - dependent protein deacetylases (class III) which are widely distributed in almost all phyla of life. Sirtuins differ from other classes of deacetylases in that they are absolutely dependent on NAD^+, deacetylates lysine residue and releases nicotinamide (NAM), 2' O-acetyl-adenosine diphosphate-ribose (AADPR) and a deacetylated substrate. As sirtuins are dependent upon the presence of NAD^+, sirtuin activity is directly linked to the metabolic state of the cell [6]. In the first step of the reaction, ADP-ribose is covalently attached to the acetyl moiety of the substrate, accompanied by the release of free NAM. Hydrolysis of the acetyl-lysine bond then occurs, liberating the AADPR. NAM acts as an inhibitor of the reaction and thus provides negative feedback inhibition of the sirtuins *in vivo* [7]. The byproduct O-acetyl ADP-ribose (AADPR) which is released in the deacetylation reaction has an essential role in modification of proteins [8,9]. Macrodomain, a conserved globular protein domain of 130-190 amino acids recognizes the terminal ADP-ribose of poly ADP ribose (PAR). Only 11 human macrodomain-containing proteins have been identified so far which are capable of binding to AADPR. Macrodomains are involved in DNA repair, transcriptional regulation, signalling events, control of NAD^+ metabolism, chromatin remodelling and developmental processes [9].

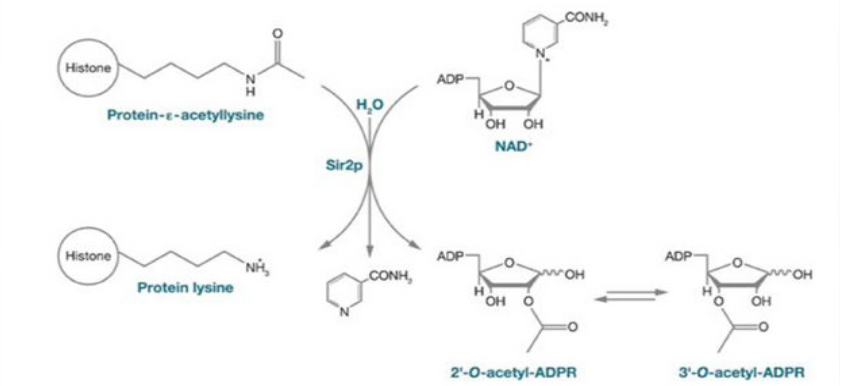

Figure 2: the sir2 reaction. Deacetylation of protien acetyllysine catalysed by sir2p. Acetyl-group transfer to the ADP-ribose (ADPR) motely of NAD* occurs via sir2p chemistry to form 2'-o-acetly-ADPR. (Adopted from sauve et al. 2006

Sirtuins are absolutely dependent on NAD^+ the abundance of free NAD^+ and its biosynthetic and breakdown products in cells are relevant to the enzymatic activity of sirtuins [10]. The root substrates for different NAD^+ biosynthetic pathways include the amino acid tryptophan (Trp), nicotinic acid, NAM and nicotinamide riboside (NR). The intermediate nicotinamide mononucleotide (NMN) can also directly stimulate NAD^+ synthesis [11]. Nicotinamide phosphoribosyltransferase (Nampt) catalysis is a rate-limiting step which transfer of phosphoribosyl group from 5-phosphoribosyl-1-pyrophosphate to nicotinamide forming NMN and pyrophosphate. The cellular levels of Nampt which synthesize NAD^+ vary during different pathophysiological conditions in metabolism like inflammation and cancer [12]. Increased expression of Nampt in response to various stresses elevates cellular NAD^+ levels, which in turn regulate catalytic activity. For example, intracellular NAD levels regulate tumour necrosis factor protein synthesis in a sirtuin-dependent manner [13].

1.2. Modulators of sirtuins

The sirtuins promote longevity in diverse species and mediate many of the beneficial effects of calorie restriction (CR), such as reduced incidence of cancer, cardiovascular disease and diabetes. Sirtuins attracted considerable interest as a therapeutic target for the development of drug targets [7]. Many inhibitors and SIRT1-activating compounds (STACs) have been discovered for sirtuins but at the molecular level, the mechanism by which sirtuins is activated remains elusive [14].

Nicotinamide which is a byproduct of the deacetylation process by sirtuins acts as an inhibitor. The synthetic molecule iso-nicotinamide (iNAM) can act as a pan-sirtuin activator within the limits of pharmacological concentrations. Several classes of plant-derived metabolites such as flavones, stilbenes, chalcones and anthocyanides activate SIRT1 *in vitro*. Resveratrol (3,5,4' – trihydroxystilbene), is the most potent of the natural activators of the sirtuins. Unfortunately, resveratrol as a drug is not likely to succeed due to insolubility, it's poor bioavailability and rapid half-life [15]. The first synthetic STACs were derivatives of an imidazothiazole scaffold (e.g. SRT460, SRT1720 and SRT2183) and chemically distinct from the polyphenol backbone of resveratrol. More potent second-generation STACs based on benzimidazole and urea-based scaffolds were subsequently described [7,16].

The sirtuin inhibitors include sirtinol (reduces inflammation in capillary endothelial cells of the skin), cambinol (competitive inhibitor that competes with acetylated polypeptides), suramin (urea derivative competes for binding with both NAD^+ and the acetylated lysine of the substrate), EX-527 (SIRT1 inhibitor, increase the acetylation of P53 protein at K382 following the induction of DNA damage in human mammary epithelial cell and some tumor cell lines), oxyindole (SIRT2 inhibitor, inhibits α-tubulin deacetylation in mammary cell lines [5].

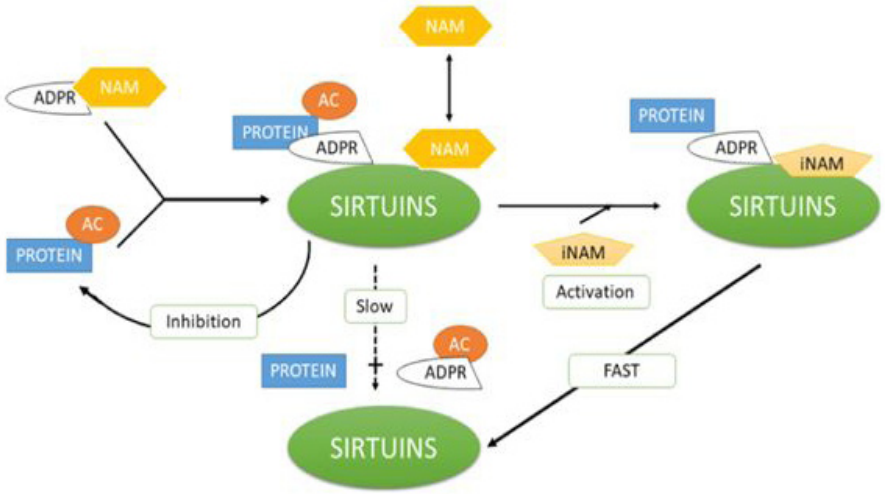

Figure 3: Activation mechanisam of sirtuins by isonicotinamide (INAM). In the presence of nicotinamide the deacetylation reaction is slow because of depletion of the imidiate by nicotinamide reactivity. isonicotinamidecan bind the nicotinamide pocket and prevent reaction reversal. The INAM-bound complex can complete deacetylation more effeciently, leading to activation of sirtuin catalytic activity in cell. (Adapted from "The Biochemistry of sirtuins" by sauve et al. 2006)

1.3. Localization of mammalian sirtuins

The seven mammalian sirtuins are nuclear encoded and ubiquitously expressed in human tissues and occupy three different subcellular localization nucleus, cytoplasm and mitochondria. SIRT 1, -2, -6, -7 are found in nucleus. SIRT1 and 2 are found in the cytoplasm as well. SIRT3, 4 and 5 are found in the mitochondria [5,17]. Sirt1 localization is predominantly nuclear but can be translocated to the cytoplasm in a cell-specific and cell-autonomous manner, in response to various physiological stimuli and disease states. The subcellular localization, enzymatic activity and the diverse functions of sirtuin isoforms are shown in **Table-1**. The various sirtuins have very diverse substrates which can be broken down into three major overlapping classes: transcriptional, apoptosis regulating and metabolic regulating. The substrates for sirtuins in *Homo sapiens* include Histones (H1, H3, H4), P53, FOXO3a, MyoD PGC-1α and other transcription factors which are deacetylated with transcription activation. In most cases, these transcription factors control genes related to growth, cell cycle and apoptosis control [10].

Table 1: Mammalian isoforms of Sirtuins, its enzyme activity and function

Sirtuin	Subcellular localization	Enzymatic activity	Function	Reference
SIRT1	Nucleus Cytoplasm	Deacetylase	• Formation of facultative and constitutive chromatin • Mitochondrial biogenesis • Fatty acid oxidation • Repress adipocyte differentiation • Regulation of cholesterol and bile acid homeostasis • Stimulate gluconeogenesis • Inhibit apoptosis	[18–26]

SIRT2	Nucleus Cytoplasm	Deacetylase Demyristoylase	• Cell cycle regulation through mitosis • Promotion of lipolysis in adipocytes • Inhibit adipocyte differentiation • Tumour suppression/promotion • Neurodegeneration	[27–33]
SIRT3	Mitochondria	Deacetylase Decrotonylase ADP-ribosyltrans-ferase	• Regulation of mitochondrial activity • Protection against oxidative stress • Enhance adaptive thermogenesis • Tumor suppression • Accelerate acetyl CoA conversion • Facilitate TCA cycle and mitochondrial energy production • Enhance fatty acid oxidation	[34–43]
SIRT4	Mitochondria	ADP-ribosyltrans-ferase Lipoamidase	• Glucose metabolism • Aminoacid catabolism • Tumor suppression • Repress aminoacid stimulated insulin	[44–48]
SIRT5	Mitochondria	Deacetylase Demalonylase Desuccinylase Deglutarylase	• Enhance urea cycle • Fatty acid metabolism • Amino acid metabolism	[49–52]
SIRT6	Nucleus	Deacetylase Deacylase ADP-ribosyltrans-ferase	• Genomic stability / DNA repair and prevent against ageing related disorders • Promote apoptosis • Reduce glycolysis and increase mitochondrial respiration • Reduce inflammatory response	[53–57]
SIRT7	Nucleus	Deacetylase	• Ribosome biogenesis • Tumor promotion	[58–61]

2. Sirtuins Mediate Effects of Calorie Restriction

There are many studies showing that sirtuins mediate the effects of calorie restriction (CR) in mammals. Restricting calorie intake, a reduction of calories by 20-40% is known as calorie restriction which can increase lifespan in lower organisms [62]. The two hallmark of CR is metabolic reprogramming (towards oxidative metabolism so as to gain most energy during the restricted diet) and resistance to stress (particularly oxidative stress) [63]. Calorie restriction induces the expression levels of SIRT1 and SIRT5 similarly, loss of function mutation of specific sirtuins ablates specific outputs of CR. However, high-fat diet leads to the loss of SIRT1 in mice [64]. The transgenic overexpression of SIRT1 or STACs mitigates disease syndrome like CR, these include diabetes, neurodegenerative diseases, liver steatosis, bone loss and inflammation [63]. Different groups of neurons in the hypothalamus control mammalian physiology & energy homeostasis, including feeding behaviour, nutrients inputs, energy expenditure, physical activity, body temperature and central circadian control.

Sirt1 is absolutely dependent on NAD+ and function as nutrient/redox sensor, and its expression is regulated by changes in nutritional status and is involved in a wide range of

metabolic processes. Nutrient sensors have the ability to sense and respond to fluctuations in environmental nutrient levels which characterize a fundamental requisite for life [65]. There are diverse nutrient sensor pathways detecting intracellular and extracellular levels of sugars, amino acids, lipids and other metabolites that incorporate and correspond at the organismal level through hormonal signals. During the period of food-richness, nutrient-sensing pathways engage in anabolism and energy storage. Conversely, shortage of food triggers homeostatic mechanisms including mobilization of internal stores through autophagy. The expression levels of SIRT1 increases upon CR in rodent and human tissues. Levels of NAD rise under CR-like conditions, which in turn induces expression of SIRT1 [66]. Thus, sirtuins act as a metabolic sensor by detecting fluctuations in the NAD^+/NADH ratio in cells. When the amount of nutrients decrease, especially in the case of glucose, levels of NAD^+ increase and sirtuins are then elevated [67]. Calorie restriction increases SIRT1 deacetylase activity in skeletal muscle in parallel with enhanced insulin-stimulated phosphoinositide 3 kinases (PI3K) signalling and glucose uptake.

3. Sirt1 in Control of Metabolic Homeostasis

The brain plays a critical role in the regulation of systemic energy homeostasis. The hypothalamus is directly sensitive to nutritional changes [68]. Among several key brain areas involved in the regulation of energy balance, the hypothalamus is the primary structure that interprets adiposity and nutrient-related inputs [69]. Among the most prominent regulators within the hypothalamus, neurons in the circumventricular organ called the arculate nucleus (ARC) of the hypothalamus located in the mediobasal hypothalamus, anteriorly juxtaposing the median eminence (ME) is of critical importance for the regulation of energy balance. Leptin is a hormone released from adipose tissue binds to leptin receptors (LEPR) on agouti-related protein (AGRP)- producing neurons and pro-opiomelanocortin (POMC)- producing neurons in the ARC of the hypothalamus [70,71]. During fasting circulating leptin levels decline rapidly. The fall in leptin stimulates the expression of AGRP and neuropeptide Y (NPY) and suppresses POMC and cocaine- and amphetamine-regulated transcript (CART), thereby increasing food intake and decreasing energy expenditure [72,73]. Recent studies have shown that SIRT1 may play a role in central energy regulatory area. For instance, it has been shown that both CR and fasting enhance SIRT1 expression and activity in the hypothalamus [69].

At the molecular level, FoxO1 is a metabolic sensor that integrates both leptin and insulin signalling. FoxO1 is the master regulator of energy metabolism across species. FoxO1 is one of the four FoxO isoforms of transcriptional factors and is highly expressed in insulin-responsive tissues including pancreas, liver, skeletal muscle and adipose tissue [74,75]. In all these tissues FoxO1 orchestrates the transcriptional cascades regulating glucose metabolism. During fasting, FoxO1 promotes adaptation by inducing gluconeogenesis in the liver and a transition from carbohydrate oxidation to lipid oxidation in the muscle. In the pancreas, FoxO1

inactivation is required for β-cell proliferation. Insulin suppresses FoxO1 activity through activation of PI3K / AKT signalling pathway. Activated AKT (also known as protein kinase B) phosphorylates FoxO1 at three highly conserved phosphorylation sites resulting in its nuclear exclusion and thus inhibition of transcription [75].

The hypothalamic mTOR (mammalian target of rapamycin) plays a role in cellular energetics by inducing numerous anabolic protein processes and lipid synthesis and it signals suppression of food intake. AMP-activated protein kinase (AMPK) is a serine/threonine kinase which is also a nutrient /energy sensor whose activity is coupled to the energy status of the cells. AMPK is activated by increased AMP/ATP ratio that occurs during fasting [76]. AMPK is involved in the regulation of numerous biochemical pathways to turn off anabolism including fatty acid degradation. AMPK is an important regulator of energy homeostasis and is stimulated by a decrease in cellular energy status, nutrient and oxygen deprivation and increased energy expenditure. Activation of AMPK results in increased expression of Nampt and supply of NAD^+ to support SIRT1 activity [77,78]. AMPK might phosphorylate SIRT1, disrupting the interaction with its negative regulator DBC1 (Deleted in Breast Cancer1, also known as KIAA1967).

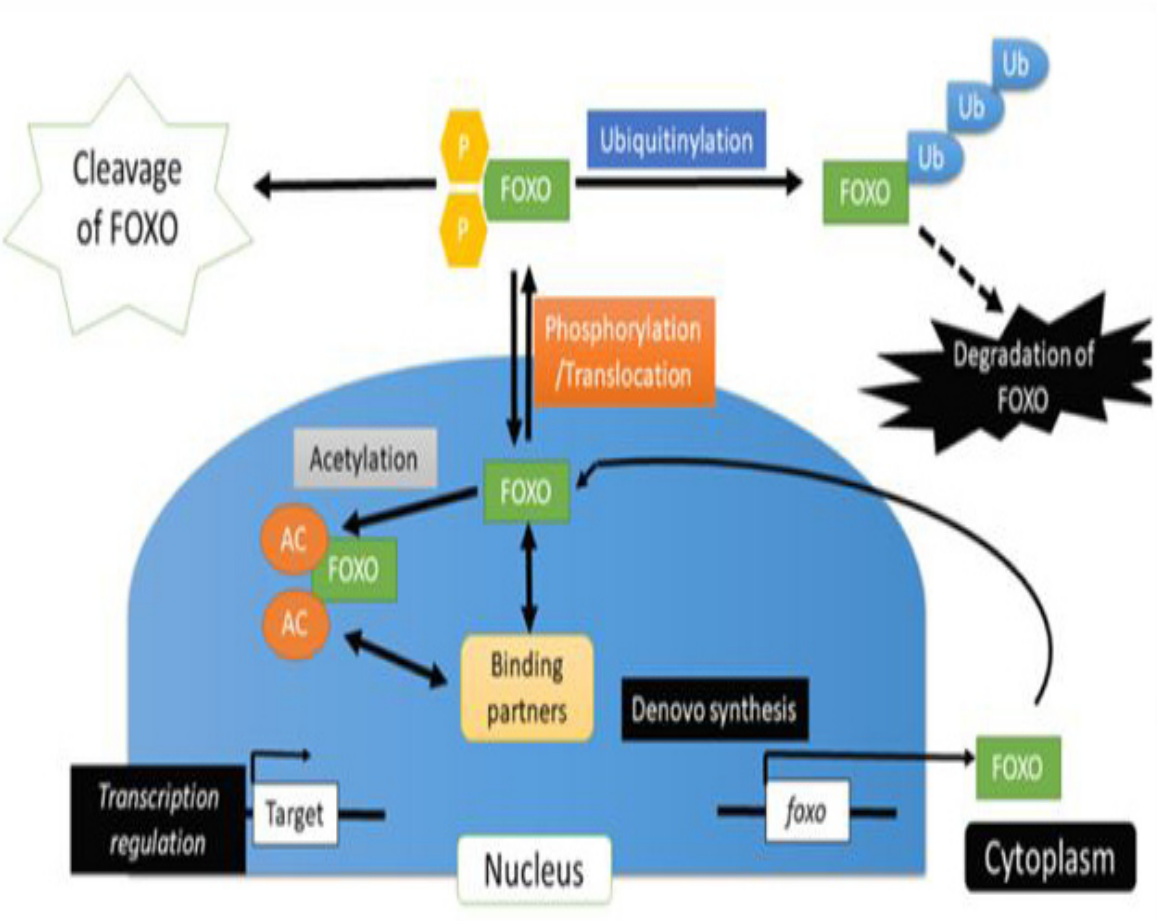

Figure 4: Mechanism regulating FOXO transcription factors. Acetylation of FOXO transcription factor is retained in the nucelus, wheresas, phosphorylation excludes the FOXO from nucleus to cytoplasm. ubiquitin dependent degradation is an irreversible step. Ub, ubiquitin; p, phosphate group; Ac, acetyl group.

3.1. Lipid metabolism

In mammals, most energy is stored as fat in adipose tissues. White adipose tissue (WAT) is the main "storage site" of excess energy, primarily in the form of triglycerides. During fasting, WAT releases fatty acids that are used as fuel by other organs [21,79]. In addition, a functionally and morphologically distinct adipocyte with dense mitochondria was found in Brown adipose tissue (BAT). Brown adipose tissue dissipates energy as heat (non-shivering thermogenesis). Brown adipocytes uncouple mitochondrial electron transport from ATP synthesis to a greater extent permeating the inner mitochondrial membrane to allow inter-membrane proton to leak back into the mitochondrial matrix, primarily through uncoupling protein-1 (UPC1) [80,81]. One of the critical regulators of fat storage in WAT is the nuclear peroxisome proliferating-activated receptor- γ (PPAR γ), whose activity promotes adipocyte differentiation and lipid anabolism. One of the suggested mechanism for loss of SIRT1 in obese animals is suggested by the finding that a high-fat diet in mice triggers cleavage of SIRT1 in WAT by caspase1 of the inflammasome [63]. The gain of function of NAD-dependent deacetylase SIRT1 or loss of function of its endogenous inhibitor DBC1 promotes "browning" of WAT by deacetylating PPAR- γ on K268 and K293 [82].

During fasting SIRT1 associated with PPAR γ and promoted the binding of the nuclear receptor corepressor1 (NCoR1). SIRT1 directly deacetylates PPAR γ which allows the recruitment of PRDM 16 to drive browning of white fat. Brown adipocytes are characterized by the expression of mitochondrial uncoupling protein 1 (UCP1), which allows dissipation of energy as heat for thermogenesis. The binding of PGC-1α to PPARγ promotes brown adipocyte-like features in white adipocytes through an upregulation of brown-adipocyte specific genes, such as UCP1, and a down-regulation of white-adipocyte specific genes. SIRT1 activation might prevent excessive accumulation of fat in adipocytes by boosting fat consumption and enhancing thermogenic function.

SIRT1 inhibits lipogenic gene expression by acting as a negative regulator of the Sterol Regulatory Element Binding Protein (SREBP)-1c. SREBP-1c is a transcription factor that promotes the expression of lipogenic and cholesterogenic genes in order to facilitate fat storage [83]. The deacetylation of SREBP-1c by SIRT1 renders the protein susceptible to ubiquitin-mediated degradation [84]. Hence, SIRT1 activation leads to decreased SREBP-1c protein levels. This results in decreased occupancy of SREBP-1c on the promoter of lipogenic genes and a concomitant reduction in their expression levels. Deacetylation of SREBP1 by SIRT1 results in targeting of SREBP1 for proteasomal degradation, which inhibits the expression of lipogenic and cholesterol synthesis genes [85]. SIRT1 also promotes reverse cholesterol transport by deacetylating and activating the LXRα nuclear receptor, and promotes the cholesterol catabolic pathway by deacetylating and activating BAR, bile acid receptor; LXRα, oxysterols receptor; SREBP-1, sterol regulatory element binding protein 1 [86,87]

Indeed, SIRT1 has been shown to modulate cholesterol metabolism *in vivo* through positive regulation of the Farnesoid X receptor (FXR) and the Liver X receptors (LRX), LXRα and LXRβ. In FXR, SIRT1 can directly deacetylate Lys 157 and Lys 217. Down-regulation of hepatic SIRT1 increases FXR acetylation, which inhibits its heterodimerization with the Retinoid X receptor (RXR) α and therefore, its transcriptional activity [88,89]. Hence, SIRT1 deletion in the liver is sufficient to downregulate FXR-related transcriptional programs and lead to the formation of cholesterol gallstones [23,86]. In LXR, ligand binding promotes the interaction with SIRT1 and subsequent deacetylation on Lys 432 (LXR α) and on Lys 433 (LXR β), promoting their activation.

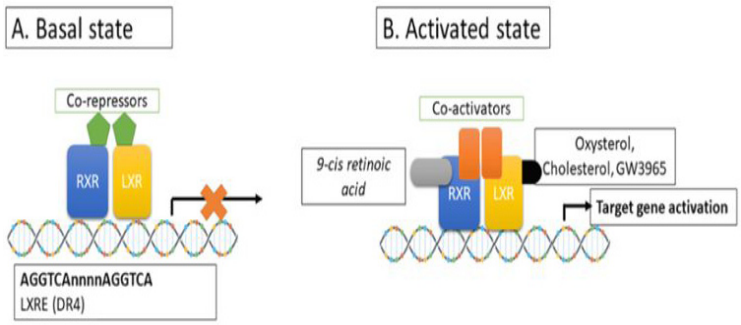

Figure 5: the liver X receptors (LXRs) and retinoid X receptors (RXRs) from heterodimer and blind to direct repeat 4 (DR4) response element in regulatory regions of their target genes. A. In basal state co-repressors are bound to heterodimer, which inhibits transcription of target genes. B. when RXR and LXR are activated by biniding of retinoic acid and oxysterols respectively the co-activators are recruited and initiate transcription of target gene.

Liver X receptor (LXR) forms an obligate heterodimer with retinoid X receptor (RXR) that binds to a DR4 (direct repeat spaced by four nucleotides) LXRE (LXR response element) in the regulatory regions of target genes, thereby repressing gene expression [86,90]. Following ligand binding to LXR or RXR, the heterodimer changes conformation, this leads to the release of corepressors and the recruitment of coactivators. This results in the transcription of target genes. Similarly, farnesoid X receptor (FXR) forms a heterodimer with RXR and binds to the FXR response element (FXRE), which is typically an inverse repeat spaced by one nucleotide (IR1), in its target genes to induce gene expression. Knockdown of SIRT1 in the liver also leads to decreased expression of CYP7A1, a bonafide LXR target. LXRs are also a potent inducer of lipid anabolism by increasing SREBP-1c activity. However, SIRT1 can deacetylate SREBP-1c resulting in proteasomal degradation [83]. The liver X receptor (LXR) controls both sterol regulatory element–binding protein (SREBP)-2 and SREBP-1c. SREBP-2 regulates the genes involved in cholesterol synthesis, while SREBP-1c stimulates the production of genes involved with the lipogenic enzymes. Inhibition of LXR would result in a decrease in both cholesterol and triglyceride synthesis [90].

Therefore, SIRT1 activation might promote the beneficial effects of LXR activity on cholesterol homeostasis while preventing the detrimental effects on lipid anabolism by deacetylating SREBP-1c. Altogether, SIRT1 overexpression improves cholesterol metabolism and prevents hepatic steatosis, while SIRT1 deletion in the liver favours lipid accumulation.

3.2. Hepatic glucose metabolism

SIRT1 participate in the energy regulation in metabolically active tissues such as liver and muscle. Gluconeogenesis during starvation can be triggered by the hormone glucagon, which induces dephosphorylation and nuclear translocation of the transducer of regulated CREB activity2 (TORC2) [91]. TORC2 activates DNA transcription factor Cre binding protein (CREB), which then induces the expression of the transcription coactivator PGC-1α. PGC-1α complexes with transcription factor PPARα, FOXO1, glucocorticoid receptor (GR) and hepatocyte nuclear factor 4α (HFN4 α) [92]. These, in turn, induce the transcription of key gluconeogenic genes encoding the rate-limiting enzymes such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [93].

In the fed state, PCG1α is phosphorylated by insulin-stimulated Akt kinase activity thereby reducing the transcription of gluconeogenic genes. At low levels of cellular ATP, in liver, AMPK inhibits gluconeogenesis by phosphorylating TORC2. AMPK activation is a key pharmacological target for developing drugs for type-II diabetes which work to suppress hepatic glucose output such as metformin [92,94]. Besides phosphorylation, PGC1 α is also regulated by acetylation and deacetylation. PGC1α is activated by SIRT1-mediated deacetylation during prolonged fasting which results in fatty acid oxidation and improved glucose homeostasis. This regulation of PGC1α activity is not dependent on glucagon or glucocorticoids but on the levels of metabolic intermediates like pyruvate and NAD. SIRT1 also affect gluconeogenic activity through PGC1α in an indirect manner. In the liver, STAT3 is known to suppress the expression of PGC1α and gluconeogenic activity gene expression. Regulation of gluconeogenesis by STAT3 could be linked to nutritional status via SIRT1, which can directly deacetylate and attenuate the anti-gluconeogenic transcriptional activity of STAT3 [25,92,95]. SIRT1 also deacetylates and activates transcriptional factor Foxo1, resulting in increased gluconeogenesis [96].

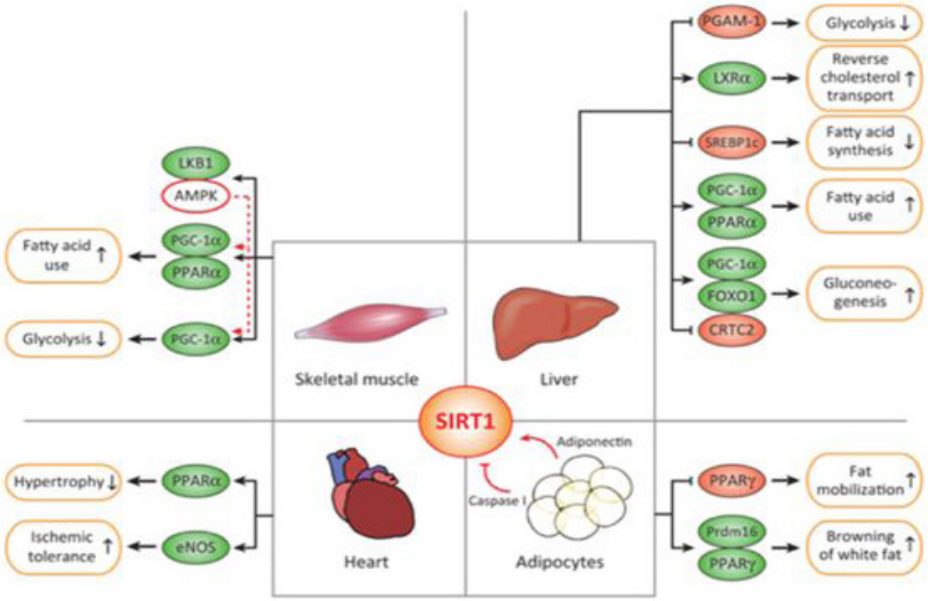

Figure 6: SIRT1 mediates metabolic benefits in various tissues. Metabolically active tissues such as liver, heart, White adipose tissue (WAT) and skeletal muscle are shown along with SIRT1 specific function. PPAR-γ (peroxisome proliferator-activated receptor, PGC-1α (PPAR-γ coactivator 1α, FOXO1 (Forkhead box O1), CRTC2 (CREB-regulated transcription coactivator 2), PGAM-1 (Enzyme phosphoglycerate mutase-1), PPARα (peroxisome proliferator-activated receptor α), SREBP1c (sterol regulatory element binding protien 1c), AMPK (AMP-activated protein kinase), NAMPT (nicotinamide phosphorilbosylt transferase), LKB1 (Liver kinase B1). Prdm16 (PR domain containing 16), eNOS (endothelial nitric oxide synthase).
Targets that are directly activated by SIRT1 are shown in Green. Those repressed or inhibited by SIRT1 are shown in pink. (Adopted from "SIRT1 and other sirtuins in metabolism" Chang et al. 2014)

4. Mitochondrial Biogenesis

SIRT1 has been described many times as a key regulator of mitochondrial biogenesis through the deacetylation of PGC-1α. PGC-1α becomes deacetylated in skeletal muscle during fasting. Similarly, PGC-1 α becomes deacetylated after exercise. Indeed, the activation of SIRT1 in response to nutrient or energy deprivation depends on AMPK activation, both *in vitro* and *in vivo*. AMPK is a master regulator of mitochondrial biogenesis and seems to play a key role in triggering SIRT1 activity during energy stress in skeletal muscle [97–99]. Cellular metabolic function controlled by mitochondrial number and function is modulated by SIRT1 through deacetylation of PPAR-co-activator 1 (PGC1). AMPK activation induces fatty acid oxidation in liver and heart, inhibits hepatic lipogenesis and adipocytes differentiation, and stimulates glucose uptake and mitochondrial biogenesis to modulate energy balance in muscle.

Insulin resistance is often characterised as the most critical factor contributing to the development of type 2 diabetes. Development of insulin resistance is a multi-step process with strong genetic and environmental influences, and the precise pathogenesis and pathophysiological sequence resulting in insulin resistance are still largely unknown. As a negative regulator of the insulin signal transduction cascade, PTP1B has been shown to function as a key insulin receptor phosphatase [100]. SIRT1 also deacetylates liver X receptor and downregulates protein tyrosine phosphatase 1B (PTP1b), a major tyrosine phosphatase for the insulin receptor and the insulin receptor substrate proteins, IRS1 and IRS2. SIRT1 was found to be required for the deacetylation and inactivation of the transcription factor STAT3 during CR, thereby promoting more efficient PI3K signalling during insulin stimulation [101]. SIRT1 could potentiate gluconeogenesis by directly deacetylating and enhancing the transcriptional activity of the Peroxisome Proliferator-activated Receptor (PPAR) gamma coactivator 1α (PGC-1α) or the Forkhead O-box protein 1 (FoxO1) transcription factor, both considered key positive controllers of the gluconeogenic transcriptional program.

5. Regulation of Circadian Rhythm

Circadian rhythm refers to an endogenous entrainable 24-hour oscillation of any biological process in all living organism. This circadian rhythm depends on internal clocks that

work in part through chromatin modification and epigenetic control of gene expression [69]. The autonomous and self-sustainable nature of circadian timing is largely dependent on the molecular circadian clockwork. Cells modify their biochemical activities depending upon the food intake and energy expenditure. This can be achieved by fine tuning the central biochemical pathway by various metabolic targets- mainly rate-limiting enzymes. The expression of these metabolic targets is modulated by the chromatin conformation and the accessibility of transcription factors that encode these enzymes. In turn, these chromatin conformations are controlled by histone acetylation, the levels of which are controlled by the concerted enzymatic activity of HATs and Histone deacetylases [102]. The circadian rhythms are directly dictated by the food availability, which is an external cue that entrains peripheral clocks. Further, several stimuli, including insulin, glucose, the glucocorticoid hormone analogue dexamethasone, forskolin and phorbol ester can trigger circadian expression *in vitro* by activating signalling cascades [103].

The molecular mechanism underlying the mammalian circadian clock consists of a transcriptional-translation feedback loop involving CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle ARNT-like1), which recognize E-box elements. CLOCK, a transcription factor is crucial for circadian function, has intrinsic histone acetyl transferase (HAT) activity [102]. The CLOCK/BMAL1 heterodimer activates the transcription of the period (Per1, 2 and 3) and cryptochrome (Cry1 and 2) leading to the subsequent repression of CLOCK/BMAL1 activity. Indeed, the cellular DNA-binding activity of CLOCK/BMAL1 is strongly influenced by the ratio of reduced to oxidized NAD cofactors. SIRT1 is a component of CLOCK/BMAL1 transcription complexes and affects the expression of clock genes [104]. SIRT1 interacts with CLOCK-BMAL1 to control the amplitude and extent of circadian clock-controlled gene expression through deacetylation of PER2 and BMAL1. Circadian regulation of SIRT1 activity is due to circadian oscillations of the cellular NAD^+ levels. It has been found that Nampt is a direct transcriptional target of CLOCK-BMAL1. Therefore, the feedback loop in the circadian clock that involves CLOCK-BMAL1, Nampt, NAD^+ and SIRT1 provide an important connection between physiological rhythm and cellular metabolism.

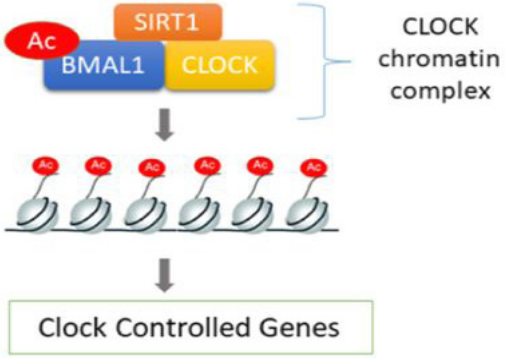

Figure 7: CLOCK is associated in a nuclear complex with BMAL1 and SIRT1, an HDAC that is directly regulated by cellular metabolism. This comples is recruited to circadian gene promoters in a cyclic manner and is thought to be responsible for the circadian acetylation of histone H3 at K9 and K14. (Adopted from "Mammalian circadian clock and metabolism-the epigenetic link" Bellet & Sassone-Corsi 2010)

6. Summary

Sirtuins are a conserved protein with NAD^+- dependent deacetylase activity and their functions are intrinsically linked to cellular metabolism. Sirtuins in lower organisms prolong the life and regulate the ageing process against metabolic stresses. The versatile functions of seven sirtuins are sustained by the distribution within the cellular compartment and tissues allowing cells to sense nutrient levels. SIRT1 plays a critical role in maintaining metabolic homeostasis with systemic effects via the hypothalamus and helps to deliver the benefits of calorie restriction. Sirtuins have numerous targets in many tissues such as liver, muscle, adipose tissue etc. which perceive the nutrient levels and respond through deacetylation of histones, key transcription factors and metabolic enzymes. SIRT1 networks with CLOCK-BMAL1 to regulate the physiological rhythm and cellular metabolism.

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