SIRT3 is a member of the sirtuin family of protein deacetylases that is preferentially localized to mitochondria. Prominent among the proteins targeted by SIRT3 are enzymes involved in energy metabolism processes, including the respiratory chain, tricarboxylic acid cycle, fatty acid β-oxidation and ketogenesis. Through these actions, SIRT3 controls the flow of mitochondrial oxidative pathways and, consequently, the rate of production of reactive oxygen species. In addition, SIRT3-mediated deacetylation activates enzymes responsible for quenching reactive oxygen species, and thereby exerts a profound protective action against oxidative stress-dependent pathologies, such as cardiac hypertrophy and neural degeneration. SIRT3 also plays a role in multiple additional metabolic processes, from acetate metabolism to brown adipose tissue thermogenesis, often by controlling mitochondrial pathways through the deacetylation of target enzymes. In general, SIRT3 activity and subsequent control of enzymes involved in energy metabolism is consistent with an overall role of protecting against age-related diseases. In fact, experimental and genetic evidence has linked SIRT3 activity with increased lifespan. In the coming years, the identification of drugs and nutrients capable of increasing SIRT3 expression or modulating SIRT3 activity can be expected to provide promising strategies for ameliorating the metabolic syndrome and other oxidative stress-related diseases that appear preferentially with aging, such as cancer, cardiac dysfunction and neural degeneration.

Key words: aging, mitochondrial, oxidative stress, SIRT3, sirtuin, thermogenesis.

SIRT3: A MITOCRHAL MEMBER OF THE SIRTUIN FAMILY

Sirtuins constitute a family of proteins with NAD⁺-dependent protein deacetylase and/or ADP-ribosyltransferase activities. Seven members of the sirtuin family (SIRT1–SIRT7) have been identified in mammals [1]. Mammalian sirtuins were first discovered based on their similarity to SIR2 (silent information regulator 2), a protein reported to be responsible for mediating the positive effects of calorie restriction on lifespan in lower organisms such as yeast, nematode worms and fruitflies [2]. Unlike traditional histone deacetylases, sirtuins deacetylate a variety of protein substrates in addition to histones, ranging from transcription factors to enzymes [3].

Although the direct link between SIR2 and the increase in lifespan due to calorie restriction has recently been questioned [4], mammalian sirtuins have been associated with many metabolic functions and have emerged as critical regulators of metabolism, influencing numerous facets of energy and nutrient homeostasis [5]. For instance, considerable evidence indicates that SIRT1, the best studied sirtuin, serves as a sensor of nutrient availability, acting on several proteins involved in the use and storage of energy in various organs and tissues [3]. To perform this function, SIRT1 activates various metabolic responses, including insulin secretion, gluconeogenesis and fatty acid oxidation, by binding and deacetylating a multitude of transcription factors. Several studies have demonstrated a protective role for SIRT1 against diseases such as glucose intolerance and fatty liver associated with chronic ingestion of a diet rich in fat [6]. However, studies in genetically modified animals suggest that, although sirtuins are related to various diseases associated with aging and mediate the positive effects of calorie restriction, they cannot by themselves increase lifespan in mammals [3,7].

Sirtuins are differentially distributed within the cell: whereas SIRT1, SIRT6 and SIRT7 are found mainly in the nucleus, and SIRT2 is primarily cytosolic, SIRT3, SIRT4 and SIRT5 are located in the mitochondria [8], an organelle that is key to cellular metabolism, owing to its central role in producing ATP, generating ROS (reactive oxygen species) and signalling during apoptosis. In agreement with a potential role in mitochondrial biology, SIRT3 is highly expressed in tissues with high oxidative capacity, such as liver, brain, kidney, skeletal muscle and BAT (brown adipose tissue) [9].

There has been some controversy surrounding the intracellular localization of SIRT3. Several studies have proposed that, in addition to mitochondria, SIRT3 may also be found in the nucleus [10–12]. One study in particular reported that human SIRT3 was a nuclear protein that was translocated to mitochondria under cellular stress conditions [11]. However, the preponderance of
evidence suggests that SIRT3 is exclusively mitochondrial [13–15]. Both human and mouse SIRT3 contain mitochondrial import signals that are cleaved during its import into mitochondria, giving rise to a shorter active form [15–17] (Figure 1).

Large-scale proteomics analyses have revealed that every major metabolic pathway contains acetylated proteins; moreover, at least 20% of mitochondrial proteins are acetylated [18]. Among these acetylated proteins are proteins belonging to a variety of metabolic pathways, such as the TCA (tricarboxylic acid) cycle, oxidative phosphorylation, fatty acid β-oxidation and the urea cycle, as well as proteins that function as mitochondrial channels or are involved in the metabolism of carbohydrates and amino acids [19]. Moreover, the global mitochondrial protein acetylation state is regulated by the nutritional status of the cell and is sensitive to fasting [18], calorie restriction [20], high-fat diet [21,22] and ethanol metabolism [23], suggesting that acetylation could be a regulatory mechanism that has evolved to provide an adaptive response to a myriad of nutritional contexts.

The study of mice with a targeted deletion of SIRT3 has been a valuable tool for clarifying the physiological role of SIRT3. One pivotal observation was a striking hyperacetylation of mitochondrial proteins in SIRT3−/− mice, a pattern not observed in SIRT4−/− or SIRT5−/− mice [24]. Moreover, incubation of mitochondrial extracts from SIRT3−/− mice with a recombinant wild-type form of SIRT3 effectively reversed this state of hyperacetylation. Collectively, these observations suggest that SIRT3 might be the main deacetylase in mitochondria. Researchers have identified several substrates of SIRT3-mediated deacetylation, demonstrating the influence of SIRT3 on multiple biological processes in which mitochondria play a relevant role.

**SIRT3 IN ENERGY METABOLISM AND ATP PRODUCTION**

A number of studies have reported that, in keeping with its localization in mitochondria, SIRT3 is involved in the control of the mitochondrial ATP-production machinery through effects on the respiratory chain. In addition to corroborating the previously reported generalized hyperacetylation of mitochondrial proteins [24], a study in SIRT3−/− mice showed abnormally reduced (nearly 50%) ATP levels in tissues that normally exhibit high ATP levels, such as heart, liver, kidney and skeletal muscle; notably, tissues where the expression of SIRT3 is normally elevated [25]. This reduction in ATP levels was not observed in tissues such as the pancreas where SIRT3 is expressed at low levels [25]. Moreover, reconstitution of SIRT3−/− MEFs (mouse embryonic fibroblasts) with wild-type SIRT3 was sufficient to fully restore ATP levels in these cells.

SIRT3−/− livers exhibit decreased oxygen consumption [25] and knockdown of SIRT3 in hepatoma cells using siRNA (small interfering RNA) partially disrupts the electron transport chain, as reflected in a decrease in mitochondrial membrane potential and elevated ROS levels [26].

It is likely that SIRT3 regulates ATP levels through the regulation of respiratory complex I activity, given that: (i) several components of complex I show increased acetylation in SIRT3−/− mice; (ii) SIRT3 can physically interact with at least one of the known subunits of complex I, the 39-kDa protein NDUFA9; and (iii) incubation of exogenous SIRT3 with mitochondria augments complex I activity [25]. In this context, SdhA (succinate dehydrogenase flavoprotein), a complex II subunit, has been reported to be a substrate of SIRT3 deacetylation [27,28]. Moreover, SIRT3−/− mice show reduced complex III and IV activity in response to a high-fat diet [22], and SIRT3 physically interacts with the proteins of respiratory complex V [28].

In addition to directly regulating the activity of the respiratory chain, SIRT3 participates in the production of reduced cofactors through activation of two enzymes: succinate dehydrogenase (as noted above) and IDH2 (isocitrate dehydrogenase 2), an NADP-dependent isoenzyme of IDH (discussed further below).

SIRT3 also participates in the flow of substrates through oxidative pathways. SIRT3−/− mice show hyperacetylation of GDH (glutamate dehydrogenase) in vivo, and SIRT3 deacetylates GDH in vitro [24]. Given that chemical acetylation of GDH reduces its activity [29], it is likely that SIRT3 might regulate GDH in vivo as well. Notably, GDH is ADP-ribosylated by SIRT4, leading to decreased GDH activity and decreased insulin secretion in response to amino acids [30]. These opposing effects of two mitochondrial sirtuins on GDH activity suggest coordinate regulation of the enzyme. This supposition is consistent with the fact that SIRT3 expression is up-regulated during calorie restriction, whereas SIRT4 activity is proposed to decline with calorie restriction to permit higher levels of GDH activity [30].

Another mechanism by which SIRT3 may regulate the synthesis of ATP, at least in the heart, is through regulation of AMPK (AMP-activated protein kinase), which acts as a sensor of cellular energy status. Once activated, AMPK activates catabolic pathways, such as glucose uptake and fatty acid oxidation to produce ATP, while inhibiting pathways that involve the expenditure of ATP. AMPK is typically activated by the regulatory kinase LKB1 (liver kinase B1). SIRT3 has been found to interact with and deacetylate (and thereby activate) LKB1 in the heart. Activated LKB1, in turn, activates AMPK, leading to high levels of ATP [31].

Finally, SIRT3 may stimulate oxidative phosphorylation indirectly by modulating the activity of CypD (cyclophilin D). Deacetylation of CypD diminishes its activity and induces its dissociation from adenine nucleotide translocator 1. This, in turn, promotes the separation of hexokinase II from the voltage-dependent anion channel in mitochondria, causing a redistribution of hexokinase II from the mitochondria to the cytosol and resulting in an increase in oxidative phosphorylation [32].

In addition to controlling mitochondrial oxidative system activity through direct interaction with enzymatic and regulatory players in mitochondrial ATP-producing pathways, SIRT3
appears to influence oxidative metabolism by controlling mitochondrial biogenesis. PGC-1α (peroxisome-proliferator-activated receptor-γ co-activator-1α) is a master controller of mitochondrial biogenesis [33]. PGC-1α induces SIRT3 expression (see below) and such induction is essential for an efficient promotion of mitochondrial biogenesis by PGC-1α [34,35].

Overall, SIRT3 appears to control the rate of mitochondrial ATP synthesis directly by controlling the activity and amount of mitochondrial respiratory and oxidative phosphorylation machinery. However, several sites for the control of catabolism by SIRT3 have been identified upstream of the respiratory chain, sites that could contribute to enhancing the provision of substrates for oxidation as a consequence of SIRT3 activation. Moreover, the control over respiratory flow by SIRT3, either direct or indirect, has consequences for ROS production that may ultimately influence multiple cellular biological processes (see below).

**SIRT3 IN ACETATE METABOLISM**

The first SIRT3 substrate identified, as described in two parallel studies published in 2006, was the mitochondrial enzyme AceCS2 (acetyl-CoA synthetase II) [36,37], which was reported to be activated by SIRT3-mediated deacetylation. AceCS2 converts acetate and CoA to acetyl-CoA. In mammals, there are two isoforms of the enzyme: AceCS1, a cytosolic form, and AceCS2, localized to the mitochondrial matrix [38]. AceCS1, which provides acetyl-CoA for the synthesis of fatty acids and cholesterol, is abundantly expressed in liver and kidney and is regulated by SIRT1. On the other hand, the mitochondrial isoform, AceCS2, produces acetyl-CoA for oxidation in the TCA cycle to produce ATP and CO2 [38]. AceCS2 is expressed mainly in BAT, heart and skeletal muscle, tissues where SIRT3 is also abundantly expressed, and it is overexpressed under ketogenic conditions.

Although the sources of acetate in mammals are diverse, the quantitative relevance of this metabolite as an energy source is just beginning to become clear. This is especially true in rodents and humans, species in which (in contrast with ruminants) acetate has not been traditionally considered to play an important role in energy metabolism [39]. Under ketogenic conditions, acetate is produced in the rodent liver as an end product of fatty acid oxidation through the hydrolysis of acetyl-CoA by the enzyme acetyl-CoA hydrolase, and is used as an alternative source of energy in peripheral tissues [40]. The main evidence for the relevance of acetate as an energy source under ketogenic conditions has come from recent studies on AceCS2−/− mice [41], in which the lack of AceCS2 enzymatic activity prevents oxidation of acetate in peripheral tissues. As a result, AceCS2−/− mice show a decrease in body temperature and less tolerance to exercise under fasting conditions. Similarly, AceCS2−/− mice show hypothermia and hypoglycaemia when fed on a high-fat low-carbohydrate diet [41].

These results suggest that acetate is an important source of energy under ketogenic conditions in non-ruminant species. As SIRT3 expression increases during calorie restriction [9], and given that SIRT3 is responsible for AceCS2 activity, SIRT3 could play a principal role in controlling the oxidation of acetate in peripheral tissues and possibly other substrates in situations of nutrient scarcity. Indeed, the phenotype of SIRT3−/− mice is similar to that of AceCS2−/− mice insofar as both are intolerant to cold exposure under fasting conditions [42]. Finally, it is worth noting that high levels of SIRT3 expression have been reported in bovine tissues, a species in which acetate is a quantitatively important source of metabolic energy [43].

Another important source of acetate in mammals is the ethanol detoxification pathway. After ethanol intake, acetate levels in human blood increase 20–30-fold [44]. Thus, through AceCS2 activation, SIRT3 could play a role in the metabolism of acetate derived from ethanol.

It is worth mentioning that studies regarding the involvement of SIRT3 in acetate metabolism are a remarkable example of how research on a novel regulatory protein may lead to the recognition of the unexpected relevance of a given metabolic pathway.

**SIRT3: A CENTRAL PLAYER IN HEPATIC METABOLISM**

**Fatty acid oxidation, ketogenesis and the urea cycle**

Several studies have indicated that SIRT3 plays a pivotal role in fatty acid oxidation in the liver, especially under conditions of a high-fat diet and during fasting, when the liver must handle fatty acids resulting from diet or mobilization of fatty acid reserves from adipose tissue.

It has been reported that SIRT3 expression is increased in the liver in response to fasting. Under these conditions, SIRT3−/− mice show an imbalance in fatty acid oxidation intermediates. Specifically, there is a greater proportion of long-chain acylcarnitines in relation to short- and medium-chain acylcarnitines in the liver and plasma [42]. Fasted SIRT3−/− mice also show a greater accumulation of hepatic triacylglycerols. However, they exhibit normal lipogenesis and fatty acid uptake by the liver; moreover, the morphology of their mitochondria is indistinguishable from that of wild-type animals. Lipid disorders observed in SIRT3−/− mice result from deficient fatty acid oxidation in the liver; such a deficiency also occurs, albeit to a lesser extent, in cardiac muscle, skeletal muscle and BAT. This oxidation deficiency is due to SIRT3-mediated deacetylation and activation of LCAD (long-chain acyl-CoA dehydrogenase), an enzyme that catalyses the oxidation of long-chain fatty acids through the fatty acid β-oxidation pathway [42]. Deficient oxidation of fatty acids may contribute to the previously described decrease in liver ATP levels in SIRT3−/− mice [25], particularly under fasting conditions.

Similar results were obtained in an independent study, which reported that SIRT3−/− mice subjected to 48 h of fasting showed increased levels of palmitoylcarnitine in the liver and of various acylcarnitines in blood, indicating deficient hepatic β-oxidation [45]. In addition, an analysis of an array of acetylated mitochondrial peptides identified several proteins that are potential targets of deacetylation by SIRT3, including some enzymes involved in fatty acid β-oxidation, such as short-chain L-3-hydroxyacyl-CoA dehydrogenase, the short/branched-chain acyl-CoA dehydrogenase, very-long-chain acyl-CoA dehydrogenase and, notably, LCAD [45].

In addition to its relevance under fasting conditions, proper fatty acid oxidation in the liver is necessary to compensate for the lipid accumulation in this tissue caused by excessive calorie intake. Ectopic fat accumulation in the liver can partially contribute to the development of the metabolic syndrome. In keeping with the potential role of SIRT3 as a critical regulator of fatty acid oxidation, enhanced lipotoxicity occurs in hepatic cells in the absence of SIRT3, as reflected in an elevated production of ROS (see below) and increased cell death in response to fatty acids. Reconstitution with wild-type SIRT3 is capable of reducing these adverse effects [26].

Recent studies established a pivotal role for SIRT3 in controlling the metabolic response to a high-fat diet. In wild-type mice, prolonged exposure to a high-fat diet is associated with a reduction in hepatic SIRT3 expression [21] and activity [22], and a general mitochondrial protein hyperacetylation pattern [21,22]. SIRT3 expression is similarly reduced in
Sirt3 acid catabolism is enhanced to sustain ketogenesis. Accordingly, activities by SIRT3 is consistent with a scenario in which fatty bodies. The co-ordinate regulation of LCAD and HMGCS2 in the liver during fasting and calorie restriction [42], may to increased activity [49]. Thus SIRT3, which is up-regulated in vitro under both basal and fasting conditions, especially the latter, in the β high-fat diet [21]. Increased levels of SCD1 are known to be associated with obesity and Type 2 diabetes [47]. Another study confirmed altered lipid oxidation in SIRT3−/− mouse liver and demonstrated that SIRT3 overexpression inhibits lipid synthesis through AMPK-dependent phosphorylation and inhibition of ACC (acetyl-CoA carboxylase), a limiting enzyme in fatty acid synthesis [48]. Acting as a sensor of cellular energy status, AMPK activates catabolic pathways while inhibiting anabolic pathways in response to a lack of ATP. The specific mechanism by which SIRT3 promotes AMPK phosphorylation has not been established, although SIRT3 can deacetyl and activate LKB1, an AMPK regulator [31], as noted above.

Taken together, these results indicate that SIRT3 plays a central role in the hepatic metabolism of fatty acids by controlling the activity of several liver proteins though regulation of their acetylation status. This regulation of fatty acid metabolism occurs at several levels: oxidation of fatty acids [21,42], subsequent use of the reducing power obtained to produce ATP in the respiratory chain [25,27], and/or inhibition of anabolic pathways of fatty acid synthesis [48]. These observations suggest that SIRT3 could be a potential therapeutic target to treat the metabolic syndrome.

Consistent with this view, a functional SNP (single-nucleotide polymorphism) (V208I) has been identified in the human SIRT3 gene that causes reduced SIRT3 activity and shown to be associated with the development of the metabolic syndrome [21].

SIRT3 is also involved in energy homeostasis during fasting, specifically controlling the production of hepatic ketone bodies. Again, comparative studies of acetylation status between SIRT3−/− and wild-type mice have shown that HMGC2S2 (3-hydroxy-3-methylglutaryl-CoA synthase 2), a limiting enzyme in the production of β-hydroxybutyrate, is deacetylated under fasting conditions in wild-type mice, but is hyperacetylated under both basal and fasting conditions, especially the latter, in the absence of SIRT3. In addition, SIRT3 can deacetylate and activate HMGC2S2 in vitro, causing a conformational change in the enzyme that leads to increased activity [49]. Thus SIRT3, which is up-regulated in the liver during fasting and calorie restriction [42], may activate HMGC2S2 and directly regulate the production of ketone bodies. The co-ordinate regulation of LCAD and HMGC2S activities by SIRT3 is consistent with a scenario in which fatty acid catabolism is enhanced to sustain ketogenesis. Accordingly, SIRT3−/− mice show a significant reduction in plasma levels of β-hydroxybutyrate under fasting conditions. Interestingly, SIRT1 deacetylates and activates HMGC51, the cytosolic isoform of the enzyme [50]. This is similar to what is observed with the cytosplasmic and mitochondrial isoforms of AceCS, and highlights the possible co-ordinated regulation by different sirtuins of certain metabolic pathways.

In addition to fatty acid oxidation and ketone body production, SIRT3 is also involved in another important metabolic pathway in the liver: the urea cycle, the key process in the detoxification of ammonia generated by amino acid catabolism. OTC (ornithine transcarbamylase), a limiting enzyme of the urea cycle, has been reported to be a substrate of SIRT3, which deacetylates Lys88 of OTC, resulting in higher enzymatic activity in vitro [45]. As a consequence, SIRT3−/− mice subjected to overnight fasting show higher hepatic levels of metabolites such as ornithine, aspartate and uridine, and decreased levels of uracil, an overall pattern typical of a dysfunctional urea cycle [45].

Interestingly, SIRT5, the other mitochondrial sirtuin with deacetylase activity, has been reported to deacetylate and activate another enzyme of the urea cycle, carbamoyl-phosphate synthase 1, under conditions of fasting, a situation in which SIRT5 is also overexpressed in the liver [51,52].

Overall, SIRT3 appears to control multiple metabolic pathways associated with an adaptive response to a shortage in energy availability, both in the liver and in extra-hepatic tissues, as depicted in Figure 2. In general, this provides a scenario in which activation of SIRT3 in liver could be envisaged for therapeutic purposes to enhance oxidative metabolism and prevent, for instance, fat accumulation. However, other lines of evidence suggesting that SIRT3 may be involved in the hepatotoxic response to drugs lessen this perspective. Lu et al. [53] recently identified ALDH2 (aldehyde dehydrogenase 2), which oxidizes aldehydes (e.g. the oxidative stress-related metabolite trans-4-hydroxy-2-nonenal) [54], as a target of SIRT3-dependent deacetylation. Under conditions in which the liver is exposed to the analgesic and antipyretic drug acetaminophen (also known as paracetamol), N-acetyl-p-benzoquinone imine, a product of acetaminophen oxidation, binds to deacetylated ALDH2, reducing its activity and ultimately aggravating acetaminophen-induced liver injury. Thus it appears that higher SIRT3 activity is associated with greater susceptibility to acetaminophen hepatotoxicity. This would help to explain the enhanced sensitivity to the toxic effects of acetaminophen under starving conditions, when SIRT3 activity in the liver is high.

**Figure 2** SIRT3 regulates multiple intracellular pathways involved in energy production, flow of substrates, and ROS production and detoxification

The main intracellular metabolic processes activated by SIRT3 are shown in blue. Enzymes and proteins directly deacetylated by SIRT3 are shown in red. For the sake of clarity, SDH and complex II are depicted separately. GPX, glutathione peroxidase; GSR, glutathione reductase; I–V, respiratory chain complexes I–V; SDH, succinate dehydrogenase.

**SIRT3 IN ADAPTIVE THERMOGENESIS AND BAT**

BAT plays a crucial role in regulating body temperature in mammals through adaptive thermogenesis. The thermogenic potential of BAT is conferred by UCP1 (uncoupling protein 1), which uncouples ATP synthesis from energy substrate oxidation...
in the respiratory chain. Adaptive thermogenesis is stimulated by β-adrenergic signalling, which regulates the intracellular enzymatic machinery for lipolysis and fuel utilization and elicits the co-ordinate transcriptional activation of genes encoding proteins specifically involved in thermogenesis, such as UCP1 [55]. A major player in the control of the thermogenic response and BAT differentiation is PGC-1α [33,56]. This transcriptional co-regulator drives the phenotypic transition of white adipocytes, which specialize in storing energy as fat, to energy-dissipating brown adipocytes [57]. It also contributes to the increase in mitochondrial biogenesis and UCP1 gene transcription in response to thermogenic activation of BAT.

SIRT3 is highly expressed in rodent BAT, where its expression is up-regulated by cold exposure [9]. SIRT3 is also expressed in association with brown adipocyte differentiation [34]. PGC-1α, a major controller of SIRT3 expression, promotes SIRT3 gene transcription through co-activation of ERα (oestrogen-related receptor-α), an orphan nuclear receptor that has been shown to bind the proximal SIRT3 gene promoter region [34]. Notably, this regulatory action of the PGC-1α–ERα axis on SIRT3 expression also takes place in other tissues, such as skeletal muscle and liver [21,35].

SIRT3 is required for the appropriate responsiveness of brown adipocytes to noradrenergic cAMP-mediated transactivation of BAT thermogenic genes. In SIRT3−/− adipocytes, PGC-1α fails to fully induce the expression of brown fat-specific thermogenic genes [34]. SIRT3 overexpression in HIB1B brown adipocytes enhances the expression of PGC-1α, UCP1 and a series of mitochondria-related genes [9].

Only a few studies have addressed thermogenic regulation in SIRT3−/− mice in vivo. Lombard et al. [24] reported that adult SIRT3−/− mice show normal adaptive thermogenesis after short-term (6 h) cold exposure. However, the combination of cold exposure and starvation was shown to have a particularly deleterious effect on metabolic homeostasis in SIRT3−/− mice [42]. During the perinatal development period, SIRT3−/− mice have a diminished capacity to induce thermogenic gene expression in BAT [58]. The possibility that compensatory mechanisms act in vivo to maintain thermogenic activity in SIRT3−/− mice in the absence of dramatic metabolic challenges in adult mice cannot be excluded. In this context, it has been reported that other sirtuins, such as SIRT1, are able to promote BAT thermogenic activity [59].

BAT activity influences body weight regulation in mice and rats owing to its high capacity for energy expenditure and fuel consumption, which is extraordinarily effective in reducing adiposity and insulin resistance [60]. The recent demonstration that active BAT is present in substantial amounts in adult humans [61], revealed by positron emission tomography technologies, has rejuvenated interest in BAT in the control of human energy balance. Moreover, a remarkable plasticity of adipose tissues in rodents has been recognized. Thus, in front of exogenous stimuli, brown adipocytes develop inside white fat depots (the so-called ‘browning’ process of adipose tissue) [62]. The current knowledge of SIRT3 as a brown-versus-white marker gene and its regulatory properties in relation to thermogenic activation warrants further research as a potential target for promotion of energy expenditure in obesity-oriented therapies.

SIRT3: A PROTECTOR OF THE HEART

SIRT3 is expressed abundantly in the heart, and has been reported to play a protective role against hypertrophy, acting at different levels.

Cardiac hypertrophy is a complex growth process in which differentiated cardiomyocytes undergo structural remodelling in response to genetic factors and to a variety of physiological and pathological stimuli. These changes provide short-term protection by diminishing wall stress and oxygen consumption, thus improving heart function. However, if the stimulus persists, hypertrophy results in the death of cardiomyocytes, fibrosis, ventricular dilatation and, eventually, heart failure and sudden death due to arrhythmias. Hypertrophic growth accompanies many forms of heart disease, including ischaemic disease, hypertension, heart failure and valvular disease. Therefore understanding the mechanisms that regulate hypertrophy is of utmost importance in efforts to improve patient survival [63].

The cardiac hypertrophic response typically involves the MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) and PI3K (phosphoinositide 3-kinase)/Akt pathways, which share upstream regulatory elements such as Ras. SIRT3 is overexpressed in the mouse heart in experimental models of phenylephrine- or angiotensin II-induced hypertrophy [64]. Moreover, SIRT3 overexpression blocks hypertrophy both in vitro (primary cultures of cardiomyocytes) and in vivo (transgenic mice overexpressing SIRT3 in the heart), whereas SIRT3−/− mice exhibit enhanced susceptibility to hypertrophy [64]. Notably, SIRT3 specifically blocks the activation of Ras in the process of hypertrophy, suggesting that the protective effect of SIRT3 in cardiac hypertrophy is mediated by the MAPK/ERK and PI3K/Akt pathways [64].

Prominent among the numerous cellular signalling mechanisms that have been implicated in the development of hypertrophy are oxidative stress and ROS production. Ras can be activated by ROS-mediated modification of the thiol group of Cys116. SIRT3 partially blocks the production of ROS in response to pro-hypertrophic stimuli by controlling the expression of genes involved in the cellular antioxidant machinery. Specifically, the mitochondrial isoform of SOD (superoxide dismutase), SOD2, and catalase are up-regulated and show higher activity in cardiomyocytes overexpressing SIRT3 and in the hearts of SIRT3 transgenic mice, whereas opposite changes occur in the hearts of SIRT3−/− mice [64]. The mechanism by which SIRT3 controls the expression of these antioxidant genes is unknown, although a role for Foxo3a (forkhead box O3A) has been suggested.

However, other mechanisms may account for the protective effects of SIRT3 on cardiac hypertrophy. Cardiac hypertrophy is associated with a reduction in the intracellular levels of NAD+, and can be blocked both in vivo and in vitro by simply adding exogenous NAD+ to maintain NAD+ intracellular levels [31]. Exogously added NAD+ blocks activation of the pro-hypertrophic Akt1 pathway and activates the anti-hypertrophic LKB1/AMPK pathway in the heart. This protective effect of NAD+ appears to be dependent on SIRT3, but not SIRT1. The SIRT3-mediated deacetylation of LKB1 and subsequent activation of AMPK may prevent the decrease in ATP and block hypertrophy [31].

In addition to cardiac hypertrophy, SIRT3 has been proposed to play a role in aging-related heart disease. Accordingly, the hearts of SIRT3−/− mice not only are more prone to cardiac hypertrophy, but also show increased sensitivity to several other diseases associated with aging, such as fibrosis and stress-related disturbances [65]. Heart failure is a major cause of death related to aging; although the causes are not understood in detail, a decrease in mitochondrial activity in the heart is known to be involved. A gradual opening of the mPTP (mitochondrial permeability transition pore), a multi-protein complex that connects the mitochondrial matrix to the cytosol, has been implicated in...
this process [66]. The opening of mPTP causes the release of pro-apoptotic factors and depletion of ATP, which can lead to apoptosis or necrosis of cardiomyocytes depending on the severity of this depletion. In this context, SIRT3 interacts with and deacetylates CypD, a regulator of mPTP opening [65], inhibiting its activity and reducing the flux through mPTP. The site in CypD deacetylated by SIRT3 (Lys165) is also adjacent to the binding site for cyclosporin A, a drug that inhibits CypD. Unfortunately, cyclosporin A also inhibits the structurally related cyclophilins CypA and CypB, which are critical for immune function [67]. As SIRT3-mediated regulation of CypD does not affect CypA or CypB function, it would inhibit mPTP activity without having a prominent effect on the immune system. Accordingly, inhibition of mPTP via SIRT3-mediated regulation of CypD could be a valuable therapeutic strategy for treating aging-associated heart disease.

As noted, cardiac hypertrophy is often closely related to oxidative stress, which causes oxidation and loss of function of many mitochondrial proteins. In fact, the transition from hypertrophy to heart failure is almost always accompanied by an increase in oxidative stress and mitochondrial dysfunction, and it is often difficult to determine which of these two processes acts primarily. SIRT3 protects against cardiac hypertrophy through the various mechanisms described above, but it also probably indirectly protects against cardiac hypertrophy by specifically controlling ROS levels, as discussed in the following section.

SIRT3 AND OXIDATIVE STRESS: A ROLE FOR SIRT3 IN AGING?

ROS generated by the leakage of electrons during electron transport in mitochondria can damage macromolecules and structures within and outside mitochondria, and are believed to be at least partially responsible for many pathologies associated with aging [68]. Several studies have highlighted the role of SIRT3 in protecting against ROS production. Notably, some of the findings of these studies provide the strongest evidence linking the activity of sirtuins with the beneficial effects of calorie restriction in mammals, particularly effects on ROS and aging. One of the benefits of calorie restriction described in higher mammals is the delay of the onset of numerous diseases associated with aging, such as cancer, diabetes, and Parkinson’s and Alzheimer’s diseases. A compelling body of evidence suggests that calorie restriction attenuates the accumulation of ROS-induced damage to different cellular macromolecules [69].

One of the pathologies associated with aging whose appearance is delayed under conditions of calorie restriction is AHL (age-related hearing loss). AHL is characterized by a progressive loss of neurons and ciliated cells of the cochlea in the inner ear due to apoptosis, which in turn causes a progressive loss of hearing. Comparative studies of wild-type and SIRT3−/− mice have shown that calorie restriction delays the onset of AHL in wild-type mice, but not in SIRT3−/− mice, indicating that SIRT3 is required for mediating the beneficial effects of calorie restriction on AHL progression in mice [70]. Consistent with this, SIRT3 expression is elevated in cells of the cochlea in response to calorie restriction. Significantly, SIRT3 is capable of preventing the accumulation of ROS under these conditions. This protective effect is due, at least in part, to the capacity of SIRT3 to deacetylate and activate IDH2, a mitochondrial enzyme that catalyses the conversion of isocitrate into 2-oxoglutarate to produce NADPH in mitochondria. Thus, under conditions of calorie restriction, SIRT3 stimulates an increase in NADPH and consequently raises the ratio of reduced glutathione/oxidized glutathione (the most important redox couple), thus protecting the cell against oxidative damage [70]. Notable in this context, SIRT3 deacetylates and activates GDH, which also produces NADPH [24,71], as noted above.

Another potential mechanism by which SIRT3 may mediate the protective effects of calorie restriction is deacetylation of the mitochondrial enzyme SOD2 [72]. Studies on SIRT3−/− mice have shown that SIRT3 protects cells from oxidative stress by activating SOD2, a major detoxificator of ROS, via deacetylation of two lysine residues, Lys53 and Lys89. Another study found that SIRT3 also activates SOD2 by deacetylating the lysine residue Lys102 [73]. Thus SIRT3 participates in controlling the levels of intracellular ROS in response to stress via post-transcriptional regulation of SOD2.

In addition to ROS detoxification, SIRT3 may directly regulate ROS production. As noted above, SIRT3 interacts with and deacetylates several components of the mitochondrial electron transport chain, including complex I and (possibly) complex III [22,25,74], which are believed to be responsible for 90% of ROS production within mitochondria. Consistent with the effects of SIRT3 on electron transport chain components, the lack of SIRT3 in muscle cells has been shown to reduce mitochondrial oxidation and increase ROS production and oxidative stress [35,74].

Notably, SIRT3 is the only sirtuin for which there is some genetic evidence for a relationship with human aging. Studies conducted in populations of southern Italy reported that a SNP (G477T) in exon three [75] and a VNTR (variable number of tandem repeats) site in intron five [76] of the human SIRT3 gene are associated with increased longevity. VNTR is associated with enhancer properties that determine the intensity of SIRT3 gene transcription, and these genetic findings are consistent with the role for SIRT3 in aging suggested by experimental data. Moreover, progressive exposure of cells and tissues to oxidative stress is considered to be a major contributor to the aging process. It appears that the role of SIRT3 in the control of ROS production may provide a functional link between the biochemical and molecular actions of SIRT3 and observational evidence of the association of SIRT3 activity with aging.

In summary, the available data from diverse tissues and organs point to the involvement of SIRT3 in both common processes (i.e. control of ROS levels) and tissue-specific functions, ranging from cardiac homoeostasis to adaptive thermogenesis in brown fat, among others. A summary of the main processes under the control of SIRT3 in different tissues and organs is depicted in Figure 3.

SIRT3 AND CANCER

The role of SIRT3 in protecting against ROS production is probably relevant to cardiac pathophysiology and aging, as mentioned above, but it may also be involved in cancer. SIRT3−/− MEFs have higher levels of ROS and superoxide radical-induced genotoxic stress, and exhibit increased chromosomal instability. Likewise, liver extracts from SIRT3−/− mice exhibit a greater degree of mitochondrial DNA damage with aging and show reduced mitochondrial integrity compared with wild-type animals. The elevated levels of ROS in SIRT3−/− mice have been proposed to contribute to a cancer-prone phenotype [77]. In addition to increased production of ROS, SIRT3−/− transformed MEFs show increased glycolysis, a characteristic of cancer cells, and decreased oxidative phosphorylation.

It has been established that transformation of primary cells in vitro requires the co-operation of at least two oncogenes, or, alternatively, simultaneous activation of a single oncogene and inactivation or deletion of a tumour-suppressor gene [78].
in vitro has been shown to eliminate the susceptibility of SIRT3 isoform of SOD involved in detoxification of intracellular ROS, a tumour suppressor. Overexpression of SOD1, the cytoplasmic α

In the absence of hydroxylation, HIF1α is stabilized and degraded. Thus aberrant stabilization of HIF1α is associated with several types of cancers [79]. In the first such study, SIRT3 overexpression was shown to be sufficient to inhibit tumorigenesis under hypoxic conditions. This effect occurred through diminished activation of HIF1α in the presence of SIRT3, reflecting the role of SIRT3 in ROS production [80]. Knockdown of SIRT3 in MEFs and various tumour lines using RNAi (RNA interference) techniques revealed that, in the absence of SIRT3, HIF1-α stability was increased and showed a greater proli erative capacity under hypoxic conditions. This effect was dependent on the increased production of ROS that occurs in the absence of SIRT3. Consistent with this, SIRT3 overexpression reduced the stability of HIF1α and decreased tumorigenesis, even when SIRT3 was overexpressed after tumour initiation [80].

A second study provided evidence that SIRT3−/− MEFs consume more glucose, have higher levels of glycolytic intermediates, lower levels of TCA cycle intermediates and exhibit hyperactivation of HIF1-α target genes, consistent with the Warburg effect characteristic of cancer cells (i.e. a shift towards glycolytic metabolism in the presence of oxygen). Notably, SIRT3 overexpression is capable of reversing the Warburg effect in several breast cancer cell lines [81]. Finally, consistent with these data, SIRT3 mice−/− are more prone to develop breast cancer, and some human tumours, such as lung cancer, have been shown to be associated with abnormally low levels of SIRT3 expression [77].

THE SIRT3 GENE: STRUCTURE AND CONTROL

The multiple roles for SIRT3 in biological processes summarized in the present review suggest that the control of SIRT3 gene expression is potentially relevant in determining the activity of SIRT3-dependent pathways in cells and tissues. As noted above, multiple reports have indicated that SIRT3 protein levels are modulated in response to physiological and hormonal stimuli, but the extent to which transcriptional compared with post-transcriptional regulatory mechanisms ultimately determine SIRT3 protein levels and activity is largely unknown. SIRT3 gene transcription appears to be modulated in a tissue-specific manner by nutrients and hormonal factors, but the mechanisms by which these regulatory processes control SIRT3 expression have not been established. However, certain features of the transcriptional regulatory region of the SIRT3 gene in mammals have been identified. In both rodents and humans, the SIRT3 5′-flanking region containing the gene promoter encompasses the PSMD13 gene encoding the p40.5 regulator subunit of the 26S proteasome. Thus the SIRT3 and PSMD13 genes are linked in a head-to-head configuration. This head-to-head organization of the two genes is evolutionarily conserved in birds, rats, mice, dogs, chimpanzees and humans, although the length of the intergenic region differs between species. For instance, in humans, this region is 788 bp long, but is only 85 bp in mice (Figure 4) [34,82]. In terms of the transcriptional control of the SIRT3 gene promoter, the only regulatory mechanism that has been reported to date is the PGC-1α-mediated mechanism involving co-activation of ERRα, described in mouse brown adipocytes, myocytes and hepatic cells [34,35], as noted above. Thus the enhanced expression of SIRT3 in association with myogenesis or brown adipocyte differentiation appears to be mediated by PGC-1α, which controls SIRT3 expression in co-ordination with an overall programme of enhanced mitochondrial biogenesis during the differentiation of these cell types. The involvement of ERRα would be consistent with the role of this nuclear receptor in the control of mitochondrial biogenesis and therefore in the coordinate expression of genes encoding mitochondrial proteins. Moreover, the VNTR intronic enhancer found in the human gene (see above) is under the control of the transcription factors GATA and AP-1 (activator protein 1) [83], and polymorphic variations at this site appear to determine variations in SIRT3 gene transcription levels that have been proposed to be associated with aging [76], as mentioned above.

CONCLUDING REMARKS

Research on sirtuins has made dramatic strides in the last decade, revealing members of this protein family to be central players in the control of major biological processes, such as aging, metabolic regulation, and the control of cell death and survival.
Although much of the focus of this research has been on SIRT1, several studies have shed increasing light on SIRT3, defining functions of this protein that are prominent among the biological actions of sirtuins. The involvement of SIRT3 in processes closely associated with human pathologies, from the metabolic syndrome to cancer, as well as in many other aging-related diseases, further highlights the ongoing interest in SIRT3 function and regulation in the context of biomedical research. As with other sirtuins, SIRT3 holds great promise as a ‘druggable protein’, i.e. a protein target capable of binding drug-like molecules. In this scenario, SIRT3 and its subsequent biological effects may be modulated by small molecules or nutrient pharmaceuticals (nutriceuticals) that influence the deacetylase activity of SIRT3. Polyphenols, such as resveratrol, have been reported to influence SIRT1 activity. At high levels, nutriceuticals such as resveratrol [84,85] and other polyphenols [86] are claimed to exert healthy beneficial effects due to SIRT3 activation, but strong scientific evidence supporting the capacity of these agents to modulate SIRT3 is still lacking. Although specific pharmacology-driven strategies to influence SIRT3 activity are still in their infancy, a number of patents relating to the development of molecules and tools to specifically modulate SIRT3 expression and/or activity, and potentially capitalize on the beneficial health effects of targeting SIRT3, have already been filed. The genuine promise of SIRT3-based pharmaceuticals must be tempered by awareness that such a mitochondria-based strategy for influencing cell processes is novel; using pharmacology to drive mitochondrial processes is still a poorly developed area. Finally, although most evidence suggests that a SIRT3 activation strategy would provide health benefits in relation to metabolism, cardiac and neural protection, and/or aging, some data show that SIRT3 activity favours hepatotoxicity [87], indicating that SIRT3 activation as therapeutic strategy should be approached with a certain degree of caution.

In summary, SIRT3 has emerged as a pivotal actor in the regulation of biological processes owing to its role in controlling enzyme activities via protein deacetylation, which appears to be a critical post-translational modification. This regulation of different pathways by SIRT3 is subject to physiological control, reflecting the context-dependent regulation of SIRT3 expression and the dependence of its deacetylase activity on NAD+ availability. The fact that SIRT3 appears to be the main sirtuin deacetylase in mitochondria, an organelle central in the control of bioenergetics, oxidative stress and cellular death processes, highlights the importance of SIRT3 and the growing recognition of its role as both a central actor in the control of basic cell biology processes and a pharmacological and/or nutritional target for intervention.

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