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Sirtuins and inflammation: friends or foes?

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Abstract

1
2 Lysine acetylation/deacetylation has been recognized as an important posttranslational
3
4 modification regulating numerous cellular processes. Sirtuins represent novel players in these
5
6 complex regulatory circuits. These NAD-dependent lysine-deacetylases have attracted much
7
8 interest based on their role in the regulation of lifespan in lower organisms, and their capacity
9
10 to interfere with cell growth, proliferation and survival in response to stress. Their absolute
11
12 requirement for NAD suggests that these enzymes may represent an important molecular link
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14 between metabolism and several human disorders such as diabetes and cancer. More recently,
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16 the identification of several transcription factors known to play a role in the immune system
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18 as sirtuin substrates has suggested that this family of enzymes may also play an important role
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20 in the regulation of inflammation, a pathological situation with clear links to metabolism and
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22 ageing in humans. We review herein the possible links between nuclear sirtuins and the
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24 regulation of an immune response, and discuss the possible strategies that may lead to the
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26 development of novel therapeutic approaches to treat inflammation by targeting sirtuin
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28 activity.
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1. Introduction

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2 The activity, stability, and intracellular location of virtually all proteins are regulated by post-
3 translational modifications (PTMs). This very diverse set of modifications include
4 phosphorylations, acetylations, sumoylations, ubiquitinations, ADP-ribosylations, nitrations
5 and many others [1]. PTMs are highly dynamic processes and most of them result from the
6 activity of antagonizing enzymes (e.g. kinases vs. phosphatases, acetylases vs. deacetylases,
7 etc). Numerous studies have highlighted the important role of PTMs in signaling pathways,
8 allowing a crosstalk between the cell and its environment and endowing the cell with a certain
9 flexibility towards change [1]. In this context, nicotinamide adenine dinucleotide (NAD) has
10 gained a renewed interest as an important substrate for a series of enzymes catalyzing a set of
11 post-translational modifications, such as deacetylation or ADP-ribosylation. In contrast to its
12 role in energy metabolism, the involvement of NAD in these regulatory processes is based on
13 its ability to act as an ADP-ribose donor, thus requiring NAD resynthesis to avoid depletion
14 of the intracellular NAD pool [2]. The central role of NAD in both energy metabolism and
15 protein modification has been shown to have important physiological consequences, in
16 particular in the control of cell metabolism, cell death and longevity [3].

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40 NAD has been linked to lifespan extension through the activity of a family of deacetylases
41 whose activity is strictly dependent on its availability [4]. NAD-dependent deacetylases, or
42 sirtuins, have indeed been shown to promote longevity in several model organisms [5],[6] and
43 in mammalian cell cultures [7], bringing NAD to the forefront of the pharmaceutical struggle
44 against aging [8]. This family comprises seven members (SIRT1 to SIRT7) that localize to the
45 nucleus (SIRT1,6 and 7), the cytoplasm (SIRT2) and the mitochondria (SIRT3,4 and5) even
46 though some of them can shuttle between these compartments. To date, SIRT4 is the only
47 member that has been shown to lack a deacetylase activity, catalyzing instead an ADP-
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ribosylation reaction [9]. The deacetylase activity of the other members of the family consists in the removal of the lysine-linked acetyl group of a target protein [10] (Figure 1). During sirtuin-mediated deacetylation, an NAD molecule is hydrolyzed, releasing a metabolite resulting from the condensation of the acetyl group with the ADP-ribose moiety of NAD (OAADPR) [10]. The other moiety of NAD, nicotinamide, is also released and acts as an end-product inhibitor of the deacetylation reaction [10]. Although sirtuins share many substrates with classical histone deacetylases (HDACs), their unique NAD-dependency links the activity of these enzymes to the intracellular concentration of available NAD, leading several authors to consider sirtuins as “metabolic sensors” [11]. Accordingly, sirtuins have been shown to regulate the cellular response to nutrient availability, intracellular glucose metabolism and cell survival in response to several forms of stress [11],[12]. The discovery that SIRT1 activity can regulate the circadian machinery [13] that potentially impacts metabolism, further argues for this sirtuin’s pivotal role in cellular metabolism, as discussed in several recently published reviews [11],[12].

(Insert Figure 1)

An additional and potentially important role for sirtuins in regulating an immune response has been recently uncovered. Several experimental observations had previously established a potential link between NAD metabolism and inflammation. In particular, expression of Nampt, the enzyme catalyzing the first and limiting reaction allowing NAD biosynthesis from nicotinamide, has been found overexpressed in several cell lines and tissues during an inflammatory response [14]. Although high rates of NAD biosynthesis may represent a biological response to meet an increased metabolic rate, this observation is also compatible with an important role for NAD-dependent enzymes in controlling immune reactions. A series of recent observations concur with this hypothesis, and clearly demonstrate a direct role for

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sirtuins in controlling the expression of several inflammatory mediators. Although a conclusive role for sirtuins as pro or anti-inflammatory regulators is still a matter of debate, several recent reports have clearly established that acetylation and sirtuin dependent deacetylation of several transcription factors with well established immunoregulatory functions, play an important role in immune cells, warranting a brief review of the literature on this new emerging field.

2. SIRT1

2.1 SIRT1 negatively regulates NFkB activity.

The NFkB transcription factor plays a pivotal role in immune function [15]. The observation that several lysines of NFkB p65 subunit can be acetylated [15] has highlighted the potential regulatory role of lysine acetylation on NFkB function. Among these, K310 acetylation confers superior transcriptional activity, while representing a substrate for SIRT1 [16]. SIRT1 acts therefore as a negative regulator of NFkB activity through the deacetylation of the p65 lysine 310. This NFkB–SIRT1 negative loop has been described in several experimental models, confirming its biological relevance. Kwon and coworkers have recently confirmed and expanded these results, by showing that this pathway is targeted and exploited by the HIV virus. The authors wished to understand the mechanism by which the HIV transcriptional activator Tat can positively regulate NFkB activity and induce IL-2 expression in infected lymphocytes [17]. In their work, they elegantly demonstrate that Tat inhibits SIRT1 enzymatic activity by binding to its acetyl-lysine-binding domain. Functional SIRT1

1 inhibition leads to NFκB hyperacetylation, which in turn causes an increase in IL-2
2 production and T cell activation, a condition that renders T cells permissive to the virus. This
3
4 SIRT1-NFκB pathway has subsequently been shown to be active in several tissues and cell
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6 types, in which a reduction in SIRT1 protein levels and/or activity is associated with an
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8 NFκB-dependent sustained inflammatory response. A decrease in SIRT1 activity has for
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10 example been reported in the lungs of smokers and of patients suffering from chronic
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12 obstructive pulmonary disease (COPD) [18], correlating with increased inflammatory gene
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14 expression and p65 K310 acetylation. An oxidative modification of the SIRT1 protein is
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16 thought to lead to increased SIRT1 decay in cells exposed to cigarette smoke [19], possibly
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18 explaining the failure to restore adequate SIRT1 activity in this model by increasing the
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20 intracellular NAD pool. [20]. In organs such as the liver or in adipose tissue that play a major
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22 role in metabolic homeostasis, SIRT1 activity can be affected by diet. In most studies, high fat
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24 diet (HFD), in which approximately 40 to 60% calories are provided from fat, is accompanied
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26 by a decrease in SIRT1 activity [21],[22], while caloric restriction (CR), that consists in a
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28 moderate caloric limitation (to approximately 60% of a standard regimen), tends to activate
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30 SIRT1 [23]. Thus SIRT1^{+/-} mice develop hepatic steatosis under a HFD, with a steatosis-
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32 associated increase in pro-inflammatory cytokine production and macrophage infiltration
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34 [24]. The same inflammatory activation is evident in SIRT1-depleted adipocytes, together
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36 with an increase in insulin resistance [22]. The putative role for NFκB in this model is
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38 supported by the observation of an NFκB hyperacetylation status [22] and by the finding that
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40 NFκB knockdown reverses these metabolic changes [22]. Importantly, free fatty acids have
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42 been shown to lead to a similar reduction in SIRT1 activity, by downregulating AMP-
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44 activated protein kinase (AMPK) [21]. This study, conducted by Yang and coworkers, shows
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46 that, like SIRT1, AMPK is a metabolic sensor that can reduce NFκB activation and that the
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48 anti-inflammatory properties of AMPK are strictly dependent on its ability to induce SIRT1-
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1 dependent p65 deacetylation [21], further supporting the physiological relevance of an
2 SIRT1-NFkB anti-inflammatory axis. Interestingly a recent report proposes that the
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4 inflammatory character of fatty acids depends on their nature: n-6 unsaturated fatty acids
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6 being pro-inflammatory while the n-3 subset has anti-inflammatory properties possibly linked
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8 to their ability to induce SIRT1 expression [25]. Besides from its dietary origin, hepatic
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10 steatosis can also be caused by excessive alcoholic absorption. Ethanol-derived metabolites
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12 have also been shown to decrease SIRT1 levels in macrophages [26]. NFkB hyperacetylation
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14 originates from this SIRT1 depletion and causes a pro-inflammatory cytokine release [26] that
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16 can reduce insulin sensitivity in co-cultured adipocytes [27]. It must be noted that
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18 inflammatory cytokines can be released by macrophages but also by non-immune cells such
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20 as hepatocytes and adipocytes. This raises the question of whether the inflammatory state
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22 observed in the liver and in the adipose tissue is of immune or hepatic/adipose origin. A
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24 similar question has been elegantly addressed by Li's team, who focused on SIRT1's role in
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26 maintaining liver homeostasis. In two successive papers, Li's team has knocked out SIRT1
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28 expression in hepatocytes [28] and in macrophages [29], and has assessed how this would
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30 affect liver steatosis following a HFD. Purushotham's liver specific SIRT1-KO mice (LKO)
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32 have impaired lipid homeostasis and are more prone to developing liver steatosis when fed a
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34 HFD, as compared to wt littermates [28]. Steatosis is accompanied by liver inflammation,
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36 with an increase in TNF α and IL-1 β synthesis and in total macrophage markers such as
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38 F4.80. However, it must be noted that Guarente's team has observed an opposite outcome in a
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40 similar model of liver-specific SIRT1 deletion, with SIRT1 absence proving to have a
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42 beneficial effect on several metabolic parameters following HFD, even if no effect on NFkB
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44 acetylation or liver inflammation was reported [30]. The increase in F4.80 expression in
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46 Purushotham's study suggests an important macrophage infiltration and this prompted the
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48 authors to disrupt SIRT1 in macrophages and assess the consequences of HFD feeding [29].
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Macrophage-specific SIRT1 deletion (Mac-SIRT1 KO) has remarkable effects on several organs and on the entire organism. HFD reduces insulin sensitivity in Mac-SIRT1 KO mice while exacerbating inflammatory cytokine expression, both systemically and locally. SIRT1 deficiency clearly correlates with NFκB hyperacetylation and NFκB knockdown blunts the increase in cytokine production observed in macrophages from SIRT1 KO mice. It is therefore tempting to conclude that, as previously stated, lack of SIRT1 activity results in hyperactive NFκB status and contributes to the development of inflammation in these models.

In line with the previous conclusion, several studies have shown that systemic SIRT1 activation decreases inflammation by reducing NFκB activity [31],[32]. SIRT1 in vivo overexpression has been achieved by Pfluger and colleagues, who generated a transgenic mouse carrying an extra copy of the SIRT1 gene under its own promoter [31]. Alternatively, SIRT1 overactivation can result from the removal of endogenous SIRT1 inhibitors, such as deleted in breast cancer-1 (DBC1) [32], a nuclear protein recently shown to bind and negatively regulate SIRT1 activity [33],[34]. Focusing on the liver, both of these studies showed that SIRT1 activation confers protection against HFD-induced steatosis and the associated inflammation. The authors also conclude that SIRT1 anti-inflammatory effects can be explained by the inhibition of NFκB, since they observe an impaired p65 activation following TNFα stimulation [31],[32].

Although numerous reports have illustrated the negative regulatory influence of SIRT1 on NF-κB, the underlying molecular mechanism has not been completely elucidated. Recently, however, two potential mechanisms by which SIRT1 can negatively affect NFκB activity have been uncovered. In a search for proteins able to modulate RelA transcriptional activity, Huang and co-workers have identified Brd4 as a bromodomain-containing protein able to specifically bind and co-activate K310-acetylated RelA [35]. Brd4 allows the recruitment of

1 the CDK9 kinase at the site of transcription, an event associated with the phosphorylation of
2 the RNA pol II [35] (Figure 2). Notably, not all NF- κ B-dependent genes are similarly
3 regulated by Brd4, suggesting that the acetylation state of RelA K310 offers a new and subtle
4 mechanism for modulating NF κ B activity. In a separate study, the same group has recently
5 demonstrated a role for the acetylation of the lysine 310 of RelA in modulating protein
6 stability [36]. Acetylation of this SIRT1 substrate impairs the Set9-mediated methylation of
7 proximal lysine residues (314 and 315), a posttranslational modification that targets
8 chromatin-associated RelA for ubiquitin-mediated degradation. Acetylated RelA is therefore
9 protected from degradation, a novel mechanism that explains the negative influence of SIRT1
10 on NF κ B function [36].
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31 **2.2 SIRT1 inhibits AP-1 by deacetylating c-Jun and c-Fos**

32 c-Jun and c-Fos are the main components of the dimeric transcription factor AP-1. In a recent
33 study, Gao and coworkers showed that c-Jun transcriptional activity can be downregulated by
34 SIRT1 in vitro [37]. A second study rapidly followed, showing that c-Jun is actively
35 deacetylated by SIRT1 in vivo [38], while a third team reported that both the subunits c-Jun
36 and c-Fos are targeted by SIRT1 [39]. These events play a major role in regulating the
37 function of several immune cells. Indeed, in macrophages, SIRT1 activation was reported to
38 decrease the transcription of COX-2, a typical AP-1 dependent pro-inflammatory gene [39].
39 In T lymphocytes, AP-1 deacetylation by SIRT1 underlies T cell anergy and permits
40 peripheral tolerance, preventing uncontrolled T cell proliferation and cytokine production
41 [38]. As a consequence, SIRT1^{-/-} mice display an increased T cell responsiveness in vitro and
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are predisposed to develop autoimmune disorders, a conclusion further confirmed by an independent study [40].

2.3 SIRT1-dependent deacetylation of FoxP3 leads to its degradation

Tregs are CD4⁺ T cells endowed with suppressive capacities, whose most specific and widely accepted marker is the transcription factor FoxP3. High and stable levels of FoxP3 expression have been proposed to be required for adequate Treg function [41]. The acetylated form of FoxP3 displays improved repressive functions and is a poor substrate for polyubiquitin-mediated degradation [42]. SIRT1 has been shown to bind and decrease FoxP3 protein levels, possibly through its deacetylation. Accordingly, nicotinamide mediated SIRT1 inhibition increases both FoxP3⁺ protein levels and the relative proportion of FoxP3⁺ cells, resulting in an overall increased Treg suppressive function [42]. In line with these results, Yang and coworkers reported that resveratrol, a natural SIRT1 activator [43], depletes CD4⁺ CD25⁺ Tregs in tumor-bearing mice [44]. As previously discussed, SIRT1 appears to limit the abundance and activity of a well described transcription factor affecting T cell functions. Notably however, and in marked contrast to the previously described observations, the antagonizing role of SIRT1 on the function and development of Tregs appears to promote, rather than inhibit, an inflammatory response.

2.4 SIRT1 role in granulopoiesis

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3 A recent report reveals that SIRT1 is not only implicated in immune cell activation but might
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5 also play an important role in their development. Indeed, Nampt-mediated NAD synthesis has
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7 been shown to trigger neutrophil differentiation from bone-marrow precursors in a SIRT1-
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9 dependent manner [45]. This pathway is affected in individuals suffering from congenital
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11 neutropenia, whose treatment consists in daily injections of granulocyte colony stimulating
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13 factor (G-CSF). Skokowa and colleagues have shown that G-CSF induces both Nampt and
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15 SIRT1 expression, leading to an increased expression of the transcription factors C-EBP α and
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17 β . C-EBP activity increases expression of both G-CSF itself and of its receptor, thus leading
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19 to a positive autoregulatory loop. This study demonstrates that administration of nicotinamide,
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21 the major NAD precursor in vivo, leads to increased NAD synthesis, C-EBP α and β
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23 expression and consequently granulopoiesis. Since C-EBP β is known to be regulated by
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25 acetylation [46], it is tempting to assume that it could be added to the growing list of
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27 transcription factors representing novel SIRT1 substrates.
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3. SIRT6

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45 Similarly to SIRT1, SIRT6 appears to be mainly localized in the nucleus, predominantly
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47 associated to chromatin [47]. In 2006 the description of SIRT6 knockout mice drew much
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49 attention due to the severe premature aging observed in these mice [47]. These mice appear
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51 normal at birth but undergo an accelerated degenerative process and die at approximately
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53 three weeks of age. They show impaired IGF1 and glucose serum levels, a decrease in bone
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55 mineral density and, at the immune level, a dramatic and systemic increase in lymphocyte
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1 apoptosis. Using these same mice, Kawahara and colleagues later demonstrated that
2 lymphopenia could be rescued by p65 heterozygosity, arguing again for a sirtuin-dependent
3 regulation of NF κ B-driven gene expression [48].
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7 **3.1. SIRT6 inhibits NF κ B dependent transcription by affecting chromatin structure.**

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10 Kawahara and colleagues focused on the possible link between SIRT6 and NF κ B to explain
11 the accelerated aging phenotype of SIRT6^{-/-} mice. These authors were able to demonstrate that
12 although SIRT6 directly binds to p65, it actually interferes with its transcriptional activity by
13 deacetylating H3 lysine 9 (H3K9) on the promoter of selected NF κ B target genes rather than
14 directly modulating p65 activity [48]. Thus, while SIRT1 inhibits NF κ B activity by direct
15 posttranslational modification of p65, SIRT6 acts by decreasing promoter accessibility to p65
16 (Figure 2). Notably, a compensatory role for SIRT6 has been recently demonstrated in SIRT1-
17 deficient macrophages [29]. In these cells, SIRT6 was found to be strongly associated with
18 selected NF κ B promoters following TNF α stimulation, thus attenuating the increased NF κ B
19 activity caused by the loss of SIRT1. Accordingly, siRNA-mediated inhibition of SIRT6
20 protein expression further increased the expression of selected NF- κ B genes (such as IL-1 β in
21 SIRT1 KO mice). Even though the nature of this compensatory mechanism was not
22 uncovered, these observations confirm that both SIRT1 and SIRT6, although acting at distinct
23 levels, represent negative regulators of NF κ B activity (Figure 2).
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45 **3.2 A potential role for SIRT6 in post-transcriptional regulation of pro-inflammatory** 46 **cytokines.**

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52 In contrast to the anti-inflammatory role generally attributed to sirtuins, we and others have
53 recently reported a possible positive regulatory role for SIRT6 in the induction of pro-
54 inflammatory cytokine expression, in both innate and adaptive immune cells [49],[50]. In
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1 these studies, reduction of intracellular NAD levels, obtained by inhibiting the enzymatic
2 activity of Nampt, led to reduced secretion of selected cytokines (such as TNF α , IL-6 and/or
3 IFN γ) by immune cells, while leaving other pro-inflammatory mediators (such as
4 CCL5/Rantes) unaffected [49],[50]. Surprisingly, several sirtuin inhibitors could reproduce
5 these effects, suggesting therefore that the NAD-sirtuin axis could also represent a positive
6 regulatory loop required for adequate cytokine secretion. Using different approaches, both
7 studies led to the identification of SIRT6 as the sirtuin member able to positively regulate
8 TNF α and IFN γ synthesis. Notably, , over-expression of an enzymatically active (but not a
9 catalytically inactive) form of SIRT6 led to supraoptimal production of TNF α by upregulating
10 the translational efficiency of TNF α mRNA [49]. In agreement with this observation,
11 downregulation of intracellular NAD levels and sirtuin inhibitors reduced TNF α protein
12 synthesis with no significant effect on TNF α mRNA accumulation in response to microbial
13 stimulation. Collectively these data suggest a complex regulatory role for SIRT6 in
14 controlling an inflammatory response, since this sirtuin member could inhibit selected NF κ B
15 target genes at the transcriptional level, while increasing the translation of other cytokine
16 mRNAs by a yet to be defined mechanism (Figure 2). Note however that TNF α gene
17 transcription does not appear to be affected by siRNA-mediated SIRT6 downregulation [29],
18 suggesting that SIRT6 may in fact specifically promote TNF α translation, while inhibiting IL-
19 1 β gene transcription in the same cells. Collectively, these observations suggest that sirtuins,
20 and in particular SIRT6, may affect the cytokine profile of activated immune cells in a subtle
21 fashion, by acting at distinct steps of the synthetic process.

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(Insert Figure 2)

4. SIRT7

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3 Finally, a function in neutrophil development has also been proposed for the nuclear sirtuin,
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5 SIRT7. Vakhrusheva and coworkers have generated a SIRT7 knockout mouse and have
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7 shown that SIRT7 disruption predisposes the mice to heart hypertrophy together with
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9 increasing cardiac inflammation [51]. These authors observed an increased infiltration of
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11 immune cells (especially granulocytes) in SIRT7^{-/-} hearts, which correlated with constitutively
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13 higher levels of in situ pro- and anti-inflammatory cytokine production, in particular in aged
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15 mice. SIRT7 has been shown to display a nucleolar localization and to affect RNA
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17 polymerase-I transcription [52]. Although this sirtuin member appears to be highly expressed
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19 by cells of the immune system (see data available at <http://biogps.gnf.org>), further work will
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21 be required to evaluate whether SIRT7 is a “bona fide” immune regulator, or whether it
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23 affects an inflammatory response indirectly by controlling immune cell survival and/or
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25 ribosomal RNA synthesis capacities.
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5. Pharmacological perspectives

5.1 Modulation of sirtuin activity through NAD metabolism.

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48 Due to their highly dynamic nature and sensitivity to small molecule modulators, enzymes
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50 mediating post-translational modifications represent ideal targets for pharmacological
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52 intervention. Sirtuins appear as particularly suitable for in vivo modulation. Their strict NAD-
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54 dependency connects these enzymes to other enzymatic pathways [53], offering numerous
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56 possibilities for affecting their enzymatic activity. Several observations indicate, for example,
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1 that increased intracellular NAD levels often lead to increased enzymatic activity of selected
2 sirtuin members [54]. This is particularly true for SIRT1, whose activity has been clearly
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4 shown to be positively regulated by protocols leading to increased intracellular NAD levels.
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6 Forced expression of Nampt in fibroblasts has been shown to increase intracellular NAD
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8 levels, causing an enhanced transcriptional repression by SIRT1. [55]. A similar direct
9
10 relationship between Nampt, intracellular NAD levels and SIRT1 activity has been recently
11
12 confirmed in several cell types including skeletal myoblasts [56], vascular smooth muscle
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14 cells [7],[57], and chondrocytes [58]. Of interest, the nuclear form of Nicotinamide
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16 Mononucleotide Adenylyl Transferase (NMNAT-1), the enzyme catalyzing the last step in
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18 NAD biosynthesis from nicotinamide in mammals, has been found to bind to SIRT1 and to be
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20 recruited to selected gene promoters [59]. This close association strongly suggests a
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22 mechanism whereby neo-synthesized NAD is made directly available to SIRT1, thus
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24 providing a link between cell metabolism and the regulation of gene transcription.
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32 The possibility to affect sirtuin activity through NAD metabolism has recently led S. Imai to
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34 propose the use of “nutriceuticals” as a novel type of sirtuin activators [8]. Although
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36 administration of NAD could represent the most straightforward approach to increase
37
38 intracellular NAD concentrations, the widespread expression of NAD-degrading ectoenzymes
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40 represent a likely mechanism affecting exogenously administered NAD bioavailability before
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42 it could reach the initially targeted cells. Moreover, systemic administration of NAD could
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44 also affect lymphocyte numbers through the process of NAD-Induced Cell Death (NICD)
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46 [60]. As previously discussed, exogenous nicotinamide, a vitamin B3 component representing
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48 the major NAD precursor in mammalian cells and displaying low toxicity in vivo [45], may
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50 represent an ideal alternative to affect intracellular NAD levels. Accordingly, SIRT1 was
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52 successfully activated in humans following oral administration of nicotinamide [45],
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1 suggesting that this strategy could prove useful in defined clinical settings. Notably however,
2 nicotinamide can both activate and inhibit sirtuins (by respectively promoting NAD synthesis
3 and acting as an end-product inhibitor of the deacetylation reaction), and it may therefore be
4 difficult to predict which of these activities will prevail in vivo. Administration of
5 nicotinamide mononucleotide (NMN), the product of the Nampt catalyzed reaction, may
6 represent a useful and clinically relevant alternative to modulate sirtuin activity in vivo [61].
7 NMN can be viewed as a rather “neutral” molecule, lacking the inhibitory properties of
8 nicotinamide and being unable to activate NAD-consuming reactions directly. Even though
9 NMN could be potentially degraded by the ectoenzyme CD38 [62], it has been shown to
10 effectively increase intracellular NAD levels in vitro, while displaying minimal toxicity when
11 administered to animals [63] Further in vivo studies are warranted to better define its
12 pharmacokinetic properties, tissue selectivity and overall effect on sirtuin activity.
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14 Inhibition of NAD-consuming enzymes represents an alternative approach to modulate sirtuin
15 activity in vivo. PARP1 (the major intracellular NAD-consuming enzyme) and SIRT1, for
16 example, are two long known rivals that share part of their substrates and whose activity
17 depends upon NAD availability, although with distinct sensitivities [64]. PARP1
18 overactivation inhibits SIRT1 activity by depleting NAD and increasing nicotinamide levels
19 [53], and PARP inhibitors have been shown to contribute to maintain high intracellular NAD
20 levels [20] resulting in increased SIRT1 activity [20],[65]. Notably, SIRT1 has also been
21 shown to directly interact with PARP1 and inactivate it in a deacetylase-dependent manner
22 [66], further illustrating the competing activities of these two NAD-consuming enzymes. In
23 any event, these preliminary observations offer new pharmacological opportunities to activate
24 sirtuins by both promoting NAD biosynthesis and by switching off competing NAD-
25 consuming enzymes.
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5.2 Small molecule modulators of sirtuin activity

The potential capacity of sirtuins to promote longevity and to increase cell resistance to stress, has led to the blossoming of numerous research activities aiming at identifying sirtuin-activating drugs. This topic has been recently reviewed (see as an example [67]) and will not be further discussed herein. It is noteworthy however that although numerous sirtuin-activating compounds have been described, their mode of action, remains often controversial. Resveratrol, the leading and most used sirtuin-activating drug [43] has been shown to affect several non-sirtuin related enzymatic activities in the cell [68], complicating the interpretation of most *in vivo* effects of this compound. Moreover, its sirtuin-enhancing capacities, originally established with an *in vitro* assay using an artificial substrate, have also been recently questioned [69]. Nonetheless, screening of several chemical libraries has led to the identification of several sirtuin-activator candidates, some of which display the expected functional properties of a bona fide sirtuin activity promoting agent. In particular, Nayagam and colleagues [70] have recently demonstrated the *in vitro* anti-inflammatory properties of SIRT1-activating compounds. *In vivo* administration of a sirtuin activator, has also been shown to decrease inflammation while concomitantly improving insulin sensitivity in naturally obese rats [27]. Finally, J. Smith and colleagues [71] have recently demonstrated the ability of two structurally distinct SIRT1 activators to recapitulate many of the signaling pathways generally controlled by SIRT1. In particular, these compounds have been shown to inhibit NF κ B transcriptional activity and TNF α secretion *in vitro*, further confirming the potential therapeutic relevance of sirtuin activators in an inflammatory setting.

Sirtuin inhibitors have also been extensively studied, mostly in the setting of cancer therapy, as shown by [72]. It is noteworthy however that nicotinamide, the prototypic sirtuin inhibitor,

1 displays anti-inflammatory properties both in vitro and in vivo [49],[73]. Although
2 nicotinamide may represent a general inhibitor for all NAD-consuming reactions, we and
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4 others have been able to confirm the potential beneficial role of sirtuin inhibitors as anti-
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6 inflammatory compounds both in vitro and in vivo [49],[50], although these observations
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8 need to be further extended to more clinically relevant settings.
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11 **Conclusions**

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19 Based on the brief review of the literature presented herein, it is tempting to conclude that
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21 sirtuins do represent clinically relevant targets for the development of novel anti-
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23 inflammatory compounds. Several complicating factors that may limit such development need
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25 however to be considered. Although numerous reports concur to identify SIRT1 as a negative
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27 regulator of NF κ B activity, it is worth noting that SIRT1 does not merely switch off NF κ B
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29 activity, but rather plays a subtle role in selectively modulating the expression of NF κ B-
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31 dependent genes. This is particularly well illustrated by the finding that SIRT1 KO mice do
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33 not display the extremely inflammatory phenotype of mice expressing a constitutively active
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35 form of the p65 NF κ B subunit [74], and by the finding that the acetylation status of the lysine
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37 310 of p65 affects the expression of some, but not all not all NF κ B target genes [35]. As a
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39 consequence, the possibility that the K310 deacetylated forms of p65 could be redirected to
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41 other target gene promoters cannot be presently excluded. As an example, in cells expressing
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43 the murine p65 mutant S276A, that is unable to recruit histone acetylases, the induction of
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45 TNF α and IL-6 is lost but the activation of the COX-2 gene is preserved [75], arguing in
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47 favor of different roles for the Lys310-acetylated and Lys310-deacetylated forms of p65.
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51 Further work is therefore required to precisely identify the target genes that may be
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59 preferentially inhibited by sirtuin activators in vivo. SIRT1 appears to negatively affect
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1 several transcription factors expressed by immune cells. In particular, the capacity of SIRT1
2 to negatively regulate the activity of both NFkB and FoxP3 appears as intriguing. When
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4 expressed by T cells, NFkB and FoxP3 regulate an immune response in opposite fashion,
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6 NFkB being considered as a positive regulator of T cell responses while FoxP3-expressing
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8 cells represent regulatory cells endowed with suppressive activity toward multiple immune
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10 effectors. This observation suggests that sirtuin inhibitors may act in a cell context dependent
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12 fashion, and although SIRT1 KO animals display an autoimmune-prone phenotype (in
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14 keeping with an overall anti-inflammatory role for SIRT1 in vivo), caution should be taken in
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16 generalizing the in vivo properties of sirtuin modulating compounds based on in vitro
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18 observations performed using purified cell subpopulations. The potential opposing roles of
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20 SIRT1 and SIRT6 on selected target genes is also a potential confounding factor illustrating
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22 the complex regulatory role of this enzyme family on the immune response. As previously
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24 discussed, although both SIRT1 and SIRT6 appear to negatively regulate the transcriptional
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26 activity of several NFkB genes, SIRT6 has also been shown to positively control the
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28 translational efficiency of TNF α mRNA. During an inflammatory reaction, TNF α is
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30 synthesized very early and several studies suggest that this cytokine orchestrates the release of
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32 other cytokines, potentially explaining the high beneficial impact of TNF α blocking reagents
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34 in the treatment of several inflammatory disorders [74]. Notably, mice expressing a
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36 constitutively active p65 form die a few weeks after birth from excessive inflammation,
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38 unless TNF α signaling is disrupted [74], We therefore believe that understanding the
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40 respective role of SIRT1 and SIRT6 in controlling TNF α production is warranted before a
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42 sirtuin-based anti-inflammatory therapy can be envisioned.
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54 Low grade, chronic inflammation is associated with aging and linked to pathological
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56 conditions, such as type 2 diabetes and cancer. Noteworthy, sirtuin activity has been shown to
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1 play a role in related processes such as cellular and organismal lifespan extension [6],[7],
2 glucose homeostasis [63] and cellular differentiation [42],[58]. The work reviewed herein
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4 suggests that some of the pathological processes linked to metabolic disorders or aging may in
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6 fact be functionally linked to the immunomodulatory function of this family of metabolic
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8 sensors.
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12 In conclusion, depending on the family member examined and on the experimental
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14 conditions, sirtuins display either pro- or anti-inflammatory properties. Although these
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16 activities can be modified by specific activators/inhibitors, a better understanding of the role
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18 of each member of the sirtuin family and the development of drugs able to specifically
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20 activate/inhibit individual sirtuins isoforms is warranted before clinically relevant applications
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22 of such compounds could be envisioned.
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Bibliography

- 1
- 2
- 3 [1] Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. *Nat*
- 4 *Struct Mol Biol* 17:666-72.
- 5 [2] Grahnert A, Grahnert A, Klein C, Schilling E, Wehrhahn J, Hauschildt S. NAD⁺: a modulator
- 6 of immune functions. *Innate Immun.*
- 7 [3] Imai S. Nicotinamide phosphoribosyltransferase (Nampt): a link between NAD biology,
- 8 metabolism, and diseases. *Curr Pharm Des* 2009;15:20-8.
- 9 [4] Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity
- 10 protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000;403:795-800.
- 11 [5] Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by
- 12 calorie restriction in *Saccharomyces cerevisiae*. *Science* 2000;289:2126-8.
- 13 [6] Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in
- 14 *Caenorhabditis elegans*. *Nature* 2001;410:227-30.
- 15 [7] Ho C, van der Veer E, Akawi O, Pickering JG. SIRT1 markedly extends replicative lifespan if
- 16 the NAD⁺ salvage pathway is enhanced. *FEBS Lett* 2009;583:3081-5.
- 17 [8] Imai S. A possibility of nutraceuticals as an anti-aging intervention: activation of sirtuins by
- 18 promoting mammalian NAD biosynthesis. *Pharmacol Res* 62:42-7.
- 19 [9] Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, et al. SIRT4
- 20 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in
- 21 pancreatic beta cells. *Cell* 2006;126:941-54.
- 22 [10] Sauve AA, Wolberger C, Schramm VL, Boeke JD. The biochemistry of sirtuins. *Annu Rev*
- 23 *Biochem* 2006;75:435-65.
- 24 [11] Yu J, Auwerx J. Protein deacetylation by SIRT1: an emerging key post-translational
- 25 modification in metabolic regulation. *Pharmacol Res* 62:35-41.
- 26 [12] Bao J, Sack MN. Protein deacetylation by sirtuins: delineating a post-translational
- 27 regulatory program responsive to nutrient and redox stressors. *Cell Mol Life Sci* 67:3073-
- 28 87.
- 29 [13] Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, et al. The NAD⁺-
- 30 dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and
- 31 circadian control. *Cell* 2008;134:329-40.
- 32 [14] Luk T, Malam Z, Marshall JC. Pre-B cell colony-enhancing factor (PBEF)/visfatin: a novel
- 33 mediator of innate immunity. *J Leukoc Biol* 2008;83:804-16.
- 34 [15] Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008;132:344-62.
- 35 [16] Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, et al. Modulation of NF-
- 36 kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *Embo J*
- 37 2004;23:2369-80.
- 38 [17] Kwon HS, Brent MM, Getachew R, Jayakumar P, Chen LF, Schnolzer M, et al. Human
- 39 immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell
- 40 hyperactivation. *Cell Host Microbe* 2008;3:158-67.
- 41 [18] Nakamaru Y, Vuppusetty C, Wada H, Milne JC, Ito M, Rossios C, et al. A protein deacetylase
- 42 SIRT1 is a negative regulator of metalloproteinase-9. *Faseb J* 2009;23:2810-9.
- 43 [19] Rajendrasozhan S, Yang SR, Kinnula VL, Rahman I. SIRT1, an antiinflammatory and
- 44 antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary
- 45 disease. *Am J Respir Crit Care Med* 2008;177:861-70.
- 46 [20] Caito S, Hwang JW, Chung S, Yao H, Sundar IK, Rahman I. PARP-1 inhibition does not
- 47 restore oxidant-mediated reduction in SIRT1 activity. *Biochem Biophys Res Commun*
- 48 392:264-70.
- 49
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- 63
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- 65

- 1 [21] Yang Z, Kahn BB, Shi H, Xue BZ. Macrophage alpha1 AMP-activated protein kinase
2 (alpha1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. *J Biol Chem*
3 285:19051-9.
- 4 [22] Yoshizaki T, Milne JC, Imamura T, Schenk S, Sonoda N, Babendure JL, et al. SIRT1 exerts
5 anti-inflammatory effects and improves insulin sensitivity in adipocytes. *Mol Cell Biol*
6 2009;29:1363-74.
- 7 [23] Guarente L, Picard F. Calorie restriction--the SIR2 connection. *Cell* 2005;120:473-82.
- 8 [24] Xu F, Gao Z, Zhang J, Rivera CA, Yin J, Weng J, et al. Lack of SIRT1 (Mammalian Sirtuin 1)
9 activity leads to liver steatosis in the SIRT1+/- mice: a role of lipid mobilization and
10 inflammation. *Endocrinology* 151:2504-14.
- 11 [25] Rahman M, Halade GV, Bhattacharya A, Fernandes G. The fat-1 transgene in mice increases
12 antioxidant potential, reduces pro-inflammatory cytokine levels, and enhances PPAR-
13 gamma and SIRT-1 expression on a calorie restricted diet. *Oxid Med Cell Longev*
14 2009;2:307-16.
- 15 [26] Shen Z, Ajmo JM, Rogers CQ, Liang X, Le L, Murr MM, et al. Role of SIRT1 in regulation of
16 LPS- or two ethanol metabolites-induced TNF-alpha production in cultured macrophage cell
17 lines. *Am J Physiol Gastrointest Liver Physiol* 2009;296:G1047-53.
- 18 [27] Yoshizaki T, Schenk S, Imamura T, Babendure JL, Sonoda N, Bae EJ, et al. SIRT1 inhibits
19 inflammatory pathways in macrophages and modulates insulin sensitivity. *Am J Physiol*
20 *Endocrinol Metab* 298:E419-28.
- 21 [28] Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of
22 SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell*
23 *Metab* 2009;9:327-38.
- 24 [29] Schug TT, Xu Q, Gao H, Peres-da-Silva A, Draper DW, Fessler MB, et al. Myeloid deletion of
25 SIRT1 induces inflammatory signaling in response to environmental stress. *Mol Cell Biol*.
26
- 27 [30] Chen D, Bruno J, Easlson E, Lin SJ, Cheng HL, Alt FW, et al. Tissue-specific regulation of SIRT1
28 by calorie restriction. *Genes Dev* 2008;22:1753-7.
- 29 [31] Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschop MH. Sirt1 protects against
30 high-fat diet-induced metabolic damage. *Proc Natl Acad Sci U S A* 2008;105:9793-8.
- 31 [32] Escande C, Chini CC, Nin V, Dykhouse KM, Novak CM, Levine J, et al. Deleted in breast
32 cancer-1 regulates SIRT1 activity and contributes to high-fat diet-induced liver steatosis in
33 mice. *J Clin Invest* 120:545-58.
- 34 [33] Zhao W, Kruse JP, Tang Y, Jung SY, Qin J, Gu W. Negative regulation of the deacetylase
35 SIRT1 by DBC1. *Nature* 2008;451:587-90.
- 36 [34] Kim JE, Chen J, Lou Z. DBC1 is a negative regulator of SIRT1. *Nature* 2008;451:583-6.
- 37 [35] Huang B, Yang XD, Zhou MM, Ozato K, Chen LF. Brd4 coactivates transcriptional activation
38 of NF-kappaB via specific binding to acetylated RelA. *Mol Cell Biol* 2009;29:1375-87.
- 39 [36] Yang XD, Tajkhorshid E, Chen LF. Functional interplay between acetylation and methylation
40 of the RelA subunit of NF-kappaB. *Mol Cell Biol* 30:2170-80.
- 41 [37] Gao Z, Ye J. Inhibition of transcriptional activity of c-JUN by SIRT1. *Biochem Biophys Res*
42 *Commun* 2008;376:793-6.
- 43 [38] Zhang J, Lee SM, Shannon S, Gao B, Chen W, Chen A, et al. The type III histone deacetylase
44 Sirt1 is essential for maintenance of T cell tolerance in mice. *J Clin Invest* 2009;119:3048-58.
- 45 [39] Zhang R, Chen HZ, Liu JJ, Jia YY, Zhang ZQ, Yang RF, et al. SIRT1 suppresses activator
46 protein-1 transcriptional activity and cyclooxygenase-2 expression in macrophages. *J Biol*
47 *Chem* 285:7097-110.
- 48 [40] Sequeira J, Boily G, Bazinet S, Saliba S, He X, Jardine K, et al. sirt1-null mice develop an
49 autoimmune-like condition. *Exp Cell Res* 2008;314:3069-74.
- 50 [41] Zhou X, Bailey-Bucktrout S, Jeker LT, Bluestone JA. Plasticity of CD4(+) FoxP3(+) T cells. *Curr*
51 *Opin Immunol* 2009;21:281-5.
- 52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1 [42] van Loosdregt J, Vercoulen Y, Guichelaar T, Gent YY, Beekman JM, van Beekum O, et al. Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. *Blood* 2003;105:965-74.
- 2
- 3 [43] Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 2003;425:191-6.
- 4
- 5 [44] Yang Y, Paik JH, Cho D, Cho JA, Kim CW. Resveratrol induces the suppression of tumor-derived CD4+CD25+ regulatory T cells. *Int Immunopharmacol* 2008;8:542-7.
- 6
- 7 [45] Skokowa J, Lan D, Thakur BK, Wang F, Gupta K, Cario G, et al. NAMPT is essential for the G-CSF-induced myeloid differentiation via a NAD(+)-sirtuin-1-dependent pathway. *Nat Med* 2009;15:151-8.
- 8
- 9 [46] Cesena TI, Cardinaux JR, Kwok R, Schwartz J. CCAAT/enhancer-binding protein (C/EBP) beta is acetylated at multiple lysines: acetylation of C/EBPbeta at lysine 39 modulates its ability to activate transcription. *J Biol Chem* 2007;282:956-67.
- 10
- 11 [47] Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 2006;124:315-29.
- 12
- 13 [48] Kawahara TL, Michishita E, Adler AS, Damian M, Berber E, Lin M, et al. SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. *Cell* 2009;136:62-74.
- 14
- 15 [49] Van Gool F, Galli M, Gueydan C, Krays V, Prevot PP, Bedalov A, et al. Intracellular NAD levels regulate tumor necrosis factor protein synthesis in a sirtuin-dependent manner. *Nat Med* 2009;15:206-10.
- 16
- 17 [50] Bruzzone S, Fruscione F, Morando S, Ferrando T, Poggi A, Garuti A, et al. Catastrophic NAD+ depletion in activated T lymphocytes through Nampt inhibition reduces demyelination and disability in EAE. *PLoS One* 2009;4:e7897.
- 18
- 19 [51] Vakhrusheva O, Smolka C, Gajawada P, Kostin S, Boettger T, Kubin T, et al. Sirt7 increases stress resistance of cardiomyocytes and prevents apoptosis and inflammatory cardiomyopathy in mice. *Circ Res* 2008;102:703-10.
- 20
- 21 [52] Ford E, Voit R, Liszt G, Magin C, Grummt I, Guarente L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev* 2006;20:1075-80.
- 22
- 23 [53] Pillai JB, Isbatan A, Imai S, Gupta MP. Poly(ADP-ribose) polymerase-1-dependent cardiac myocyte cell death during heart failure is mediated by NAD+ depletion and reduced Sir2alpha deacetylase activity. *J Biol Chem* 2005;280:43121-30.
- 24
- 25 [54] Yang H, Yang T, Baur JA, Perez E, Matsui T, Carmona JJ, et al. Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. *Cell* 2007;130:1095-107.
- 26
- 27 [55] Revollo JR, Grimm AA, Imai S. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. *J Biol Chem* 2004;279:50754-63.
- 28
- 29 [56] Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, Sauve AA, et al. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev Cell* 2008;14:661-73.
- 30
- 31 [57] van der Veer E, Nong Z, O'Neil C, Urquhart B, Freeman D, Pickering JG. Pre-B-cell colony-enhancing factor regulates NAD+-dependent protein deacetylase activity and promotes vascular smooth muscle cell maturation. *Circ Res* 2005;97:25-34.
- 32
- 33 [58] Dvir-Ginzberg M, Gagarina V, Lee EJ, Hall DJ. Regulation of cartilage-specific gene expression in human chondrocytes by SirT1 and nicotinamide phosphoribosyltransferase. *J Biol Chem* 2008;283:36300-10.
- 34
- 35 [59] Zhang T, Berrocal JG, Frizzell KM, Gamble MJ, DuMond ME, Krishnakumar R, et al. Enzymes in the NAD+ salvage pathway regulate SIRT1 activity at target gene promoters. *J Biol Chem* 2009;284:20408-17.
- 36
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- 38
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- 40
- 41
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46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [60] Adriouch S, Hubert S, Pechberty S, Koch-Nolte F, Haag F, Seman M. NAD⁺ released during inflammation participates in T cell homeostasis by inducing ART2-mediated death of naive T cells in vivo. *J Immunol* 2007;179:186-94.
- [61] Imai S. The NAD World: a new systemic regulatory network for metabolism and aging-- Sirt1, systemic NAD biosynthesis, and their importance. *Cell Biochem Biophys* 2009;53:65-74.
- [62] Sauve AA, Munshi C, Lee HC, Schramm VL. The reaction mechanism for CD38. A single intermediate is responsible for cyclization, hydrolysis, and base-exchange chemistries. *Biochemistry* 1998;37:13239-49.
- [63] Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A, et al. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab* 2007;6:363-75.
- [64] Zhang J. Are poly(ADP-ribosyl)ation by PARP-1 and deacetylation by Sir2 linked? *Bioessays* 2003;25:808-14.
- [65] Hwang JW, Chung S, Sundar IK, Yao H, Arunachalam G, McBurney MW, et al. Cigarette smoke-induced autophagy is regulated by SIRT1-PARP-1-dependent mechanism: implication in pathogenesis of COPD. *Arch Biochem Biophys* 500:203-9.
- [66] Rajamohan SB, Pillai VB, Gupta M, Sundaresan NR, Birukov KG, Samant S, et al. SIRT1 promotes cell survival under stress by deacetylation-dependent deactivation of poly(ADP-ribose) polymerase 1. *Mol Cell Biol* 2009;29:4116-29.
- [67] Alcain FJ MR, Villalba JM, de Cabo R Chapter 10 Small Molecule Modulators of Sirtuin Activity. In: GM F, editor. *The Future of Aging*: Springer, 2010.
- [68] Mader I, Wabitsch M, Debatin KM, Fischer-Posovszky P, Fulda S. Identification of a novel proapoptotic function of resveratrol in fat cells: SIRT1-independent sensitization to TRAIL-induced apoptosis. *Faseb J* 24:1997-2009.
- [69] Pacholec M, Bleasdale JE, Chrnyk B, Cunningham D, Flynn D, Garofalo RS, et al. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. *J Biol Chem* 285:8340-51.
- [70] Nayagam VM, Wang X, Tan YC, Poulsen A, Goh KC, Ng T, et al. SIRT1 modulating compounds from high-throughput screening as anti-inflammatory and insulin-sensitizing agents. *J Biomol Screen* 2006;11:959-67.
- [71] Smith JJ, Kenney RD, Gagne DJ, Frushour BP, Ladd W, Galonek HL, et al. Small molecule activators of SIRT1 replicate signaling pathways triggered by calorie restriction in vivo. *BMC Syst Biol* 2009;3:31.
- [72] Heltweg B, Gatbonton T, Schuler AD, Posakony J, Li H, Goehle S, et al. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res* 2006;66:4368-77.
- [73] Ungerstedt JS, Blomback M, Soderstrom T. Nicotinamide is a potent inhibitor of proinflammatory cytokines. *Clin Exp Immunol* 2003;131:48-52.
- [74] Dong J, Jimi E, Zeiss C, Hayden MS, Ghosh S. Constitutively active NF-kappaB triggers systemic TNFalpha-dependent inflammation and localized TNFalpha-independent inflammatory disease. *Genes Dev* 24:1709-17.
- [75] Dong J, Jimi E, Zhong H, Hayden MS, Ghosh S. Repression of gene expression by unphosphorylated NF-kappaB p65 through epigenetic mechanisms. *Genes Dev* 2008;22:1159-73.

Figure Legends

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5 **Figure 1:** Schematic representation of nicotinamide recycling to NAD and of the reaction
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7 catalyzed by sirtuins. Nicotinamide (Nam) is the major NAD precursors for mammalian cells
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9 both in vivo and in vitro. It can be converted to NAD through two successive enzymatic
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11 reactions: the first one is rate-limiting and is catalyzed by nicotinamide phosphoribosyl
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13 transferase (Nampt). Nicotinamide mononucleotide arises from this reaction and is converted
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15 to NAD by nicotinamide/nicotinic acid mononucleotide adenylyl transferase. In the presence
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17 of NAD, sirtuins can remove the acetyl group on specific lysines of a given substrate and
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19 transfer it on the ADP ribose moiety of an NAD molecule. The reaction also releases the
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21 deacetylated substrate and nicotinamide that can be recycled to NAD. If nicotinamide is not
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23 cleared, however, it can inhibit the deacetylation reaction. This reaction can also be indirectly
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25 impaired by the activation of members of the PARP family that deplete the NAD cellular pool
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27 and contribute to nicotinamide accumulation.
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39 **Figure 2:** Putative model of how two NAD-dependent deacetylases, SIRT1 and SIRT6, can
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41 modulate the expression of pro-inflammatory mediators, by concerted or opposing
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43 mechanisms. SIRT1 deacetylation of p65 lysine 310 can inhibit the recruitment of the
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45 bromodomain-containing coactivator Brd4. Lack of Brd4 recruitment is thought to impair the
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47 binding of CDK9 and the recruitment and phosphorylation of RNA polymerase II (pol II),
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49 leading to reduced transcription of several pro-inflammatory mediators such as interleukin
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51 (IL) -1 β , -2, -6, TNF α and MMP9. An additional mechanism could reduce IL-1 β transcription
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53 through SIRT6-dependent deacetylation of histone 3 lysine 9 in nucleosomes. Whether this
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55 mechanism exclusively regulates this cytokine expression remains to be established. Finally,
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1 SirT6 could also have a positive effect on the translation of TNF α mRNA and, possibly, other
2 selected transcripts. Note that SirT6 activity is either exclusively positive (e.g. TNF α) or
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5 negative (e.g. IL-1 β) for a given pro-inflammatory gene expression.
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Figure

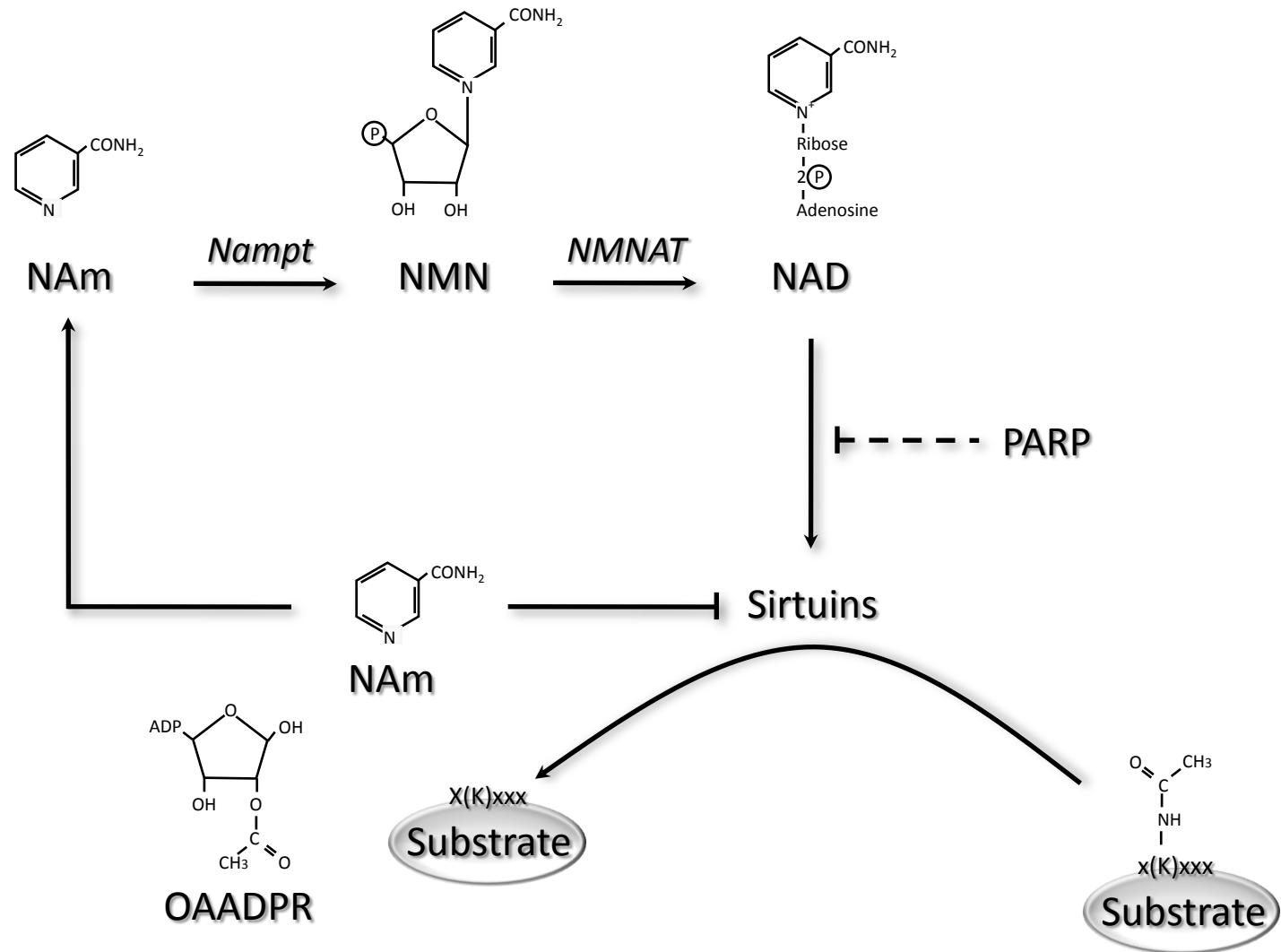


Figure 1

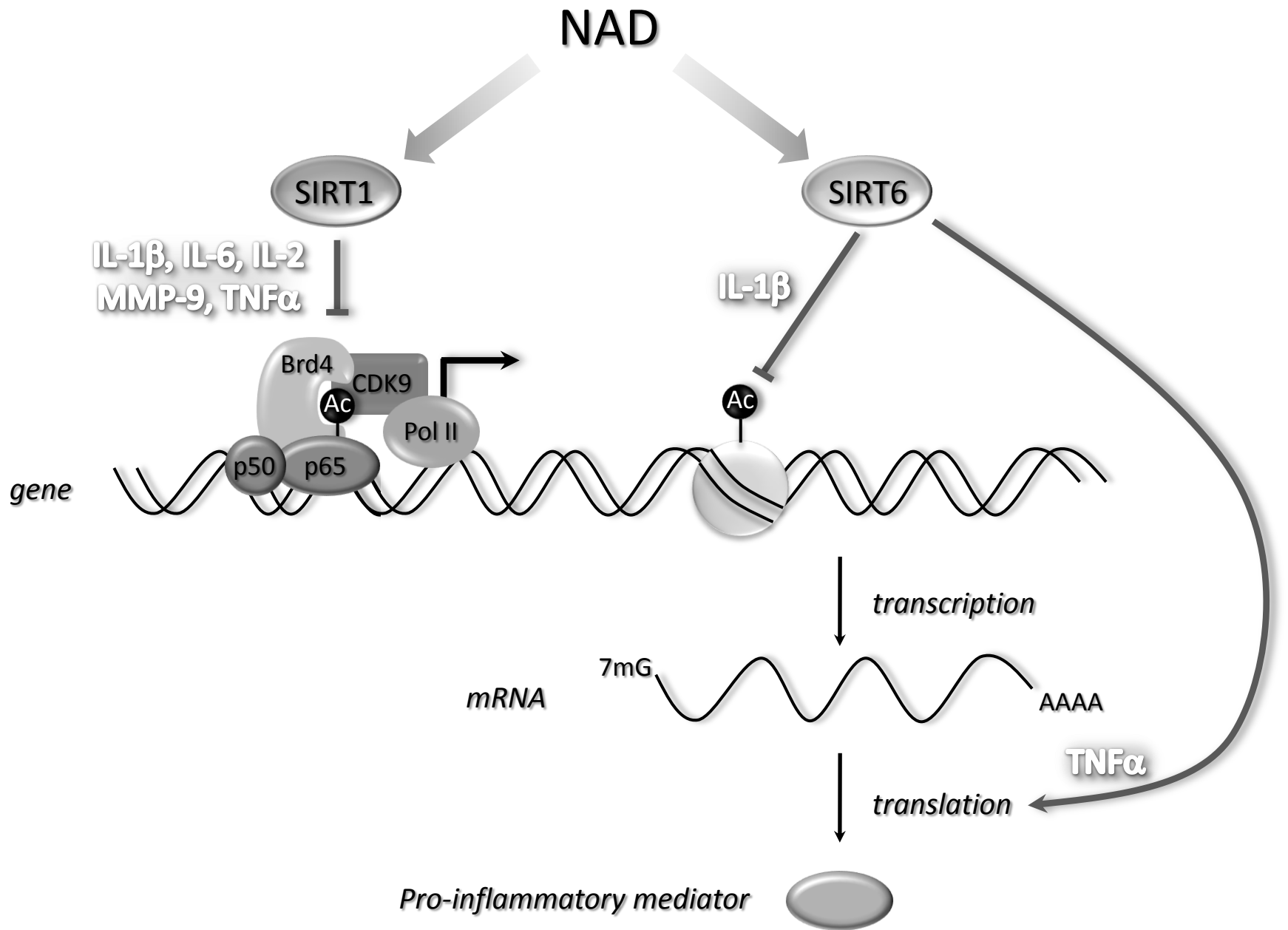


Figure 2