

A new facet of ADP-ribosylation reactions: SIRT6 and PARPs interplay

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. The family of Sirtuins
4. The family of PARPs
5. The interplay of Sirtuins and PARPs
6. Concluding remarks
7. Acknowledgements
8. References

1. ABSTRACT

Nicotinamide Adenine Dinucleotide (NAD⁺) is known mainly as coenzyme of redox reactions for energy transduction and is consumed as substrate in regulatory reactions removing nicotinamide and producing ADP-ribose. Several families of ADP-ribose synthesizing enzymes use NAD⁺ as substrate and control processes like DNA repair, replication and transcription, chromatin structure, the activity of G-proteins and others. Since NAD⁺-dependent reactions involve degradation of the dinucleotide, a constant supply of the pyridinic substrate is required for its homeostasis. NAD⁺-dependent signaling reactions include protein deacetylation by sirtuins, intracellular calcium signaling and mono-/poly-ADP-ribosylation. In the context of all NAD⁺-dependent reactions leading to ADP-ribose synthesis, this review focuses mainly on both the central role played by sirtuins and poly-ADP-ribose polymerases as cellular NAD⁺ consumers and their crosstalk in signaling pathways.

2. INTRODUCTION

Nicotinamide Adenine Dinucleotide (NAD⁺) plays a major role in all cells as cofactor in metabolic redox reactions and as substrate for signal transduction that is regulated by NAD⁺ concentration (1-5). The cleavage of NAD⁺ leads to the synthesis of poly-, mono-, cyclic- and O-acetyl-(ADP-ribose) structures in reactions that control processes such as DNA repair, chromatin structure, telomere stability, chromosome sorting,

G-protein activity and neuronal calcium signaling, widely described elsewhere (6-9). A decrease of NAD⁺ levels can have severe consequences. NAD⁺ deprivation has been associated with major human diseases such as age- and diet-induced disorders, neurodegeneration and cancer (10-14). This review focuses on both the central role played by sirtuins and poly-ADP-ribose polymerases as main cellular NAD⁺ consumers and their crosstalk in signaling pathways.

Sirtuins are a family of NAD⁺-dependent protein deacetylases widely distributed in all phyla of life. The use of NAD⁺ as a co-substrate distinguishes sirtuins from other classes of protein deacetylases. Accumulating evidence indicates that sirtuins are important regulators of organism life span (10, 15, 16). Still debated is the ADP-ribosyltransferase activity of some sirtuins (10).

Protein ADP-ribosyltransferases catalyze the transfer of adenosine diphosphate ribose (ADP-ribose) from nicotinamide adenine dinucleotide (NAD) onto specific target proteins (9). The activity of PARP family members is involved in cellular signalling pathways through a complex response that is driven by mono- (MAR) and poly(ADP-ribose) (PAR) (8, 17-20). Removal of ADP-ribose units from PARP-1 and other proteins is mediated by PARG (21) and ARH3 (22, 23) which promote rapid degradation and turnover of PAR polymers. The cross-talk among different PARPs suggests specific functions for each

Table 1. Mammalian sirtuins

Sirtuin	Enzyme Activity	Main Substrates	Role in	Cell localization
SIRT1	Deacetylase	p53, Foxo1, Foxo3, Bax, Hif-1 alpha, Hif-2a, HSF1, Ku70, b-catenin, E2F1, Myc, STAT3, PGC-1 alpha, NF-kB, TORC2, LXR, FXR, SREBP, PER2, CLOCK	Energy metabolism, stress response	Nucleus Cytoplasm
SIRT2	Deacetylase	Tubulin, PEPCK, Foxo1, PAR3	Cell cycle	Cytoplasm
SIRT3	Deacetylase	Oxidative P-rylation complex I, AceCS2, LCAD, HMG-CoA synthase 2, IDH2, MnSOD, SOD2	ATP synthesis, anti-oxidativestress, thermogenesis	Mitochondria
SIRT4	ADPR-transferase	GDH	Insulin secretion, fatty acid oxidation	Mitochondria
SIRT5	Deacetylase	CPS1	Urea cycle	Mitochondria
SIRT6	Deacetylase/ ADPR-transferase	H3K9, H3K56, CtIP, SIRT6	DNA repair, metabolism, inflammation	Nucleus
SIRT7	Deacetylase?	p53?	rDNA transcription	Nucleolus

CLOCK, Circadian Locomotor Output Cycles Kaput; CPS1, carbamoyl phosphate synthetase 1; FOXO, forkhead box O; FXR, farnesoid X receptor; GDH, glutamate dehydrogenase; HIF1alpha, hypoxia-inducible factor 1α; HMGCS2, 3-hydroxy-3-methylglutaryl CoA synthase 2; IDH2, isocitrate dehydrogenase 2; LCAD, long-chain acyl CoA dehydrogenase; LXR, liver X receptor; NF-kB, nuclear factor-kB; OXPHOS, oxidative phosphorylation; PAR3, partitioning defective 3 homologue; PEPCK, phosphoenolpyruvate carboxykinase; PER2, PERiod circadian protein homolog 2; PGC1alpha, peroxisome proliferator-activated receptor-γ co-activator 1alpha; SIRT, sirtuin; SOD2, superoxide dismutase 2; SREBP1c, sterol-response element-binding protein 1c; TORC2, Transducer Of Regulated CREB 2

PARP family member (17, 19, 20). The increase of these members in the evolutionary scale up to 17 enzymes in mammals, accounts for specialized roles. These functions are further diversified by the expression and localization patterns of individual PARPs (9).

Cross-talking has been described between sirtuins and PARPs too (24, 25). New information are available regarding PARP-1 and sirtuins regulating various physiological processes as well as cell death and ischemic brain damage (25). All together these findings support the hypothesis that NAD⁺ metabolism, PARP-1 and sirtuins play fundamental roles and are involved in metabolism, oxidative stress and ageing (10, 25).

This review is a survey of the most recent findings on the interplay of sirtuins, particularly SIRT 1, and PARPs, mainly PARP 1 and 2, mediated by cellular NAD⁺ concentration.

3. THE FAMILY OF SIRTUINS

Sirtuins are highly conserved NAD⁺-dependent protein deacetylases and/or ADP-ribosyltransferases that can extend the lifespan of several lower model organisms including yeast, worms and flies (10, 26). Yeast SIR2 was the first discovered NAD⁺-dependent histone

deacetylase (27, 28). Thereafter seven sirtuins (SIRT 1-7) were identified in mammals, with mammalian SIRT1 evolutionarily the closest to yeast Sir2 (29, 30). The mammalian sirtuins have been connected to various activities from cellular stress resistance and genomic stability, to tumorigenesis and energy metabolism (10). All mammalian sirtuins are in various cell compartments, have a conserved NAD⁺-binding and catalytic domain (sirtuin core domain), have different N- and C-terminal domains, and differ for substrate specificity (histones and non-histones), and biological functions (Table I) (26). Yeast and mammalian sirtuins catalyze the same reaction, NAD⁺-dependent histone deacetylation (Figure 1) (31, 32). The two-step deacetylation reaction of sirtuins starts with the cleavage of NAD⁺ at the beta-N glycosidic linkage to remove nicotinamide (NAM), followed by the transfer of the acetyl group from the substrate to the ADP-ribose moiety to form O-acetyl-ADP ribose and the deacetylated substrate (33, 34). Despite different subcellular localizations and a broad range of substrate specificities, the activity of all sirtuins is directly controlled by cellular NAD⁺ levels, which are an indicator of cellular metabolic status. The activity of these enzymes is also inhibited by their common enzymatic product, nicotinamide (35).

The seven mammalian sirtuins, SIRT1 to SIRT7, have emerged as key metabolic sensors that

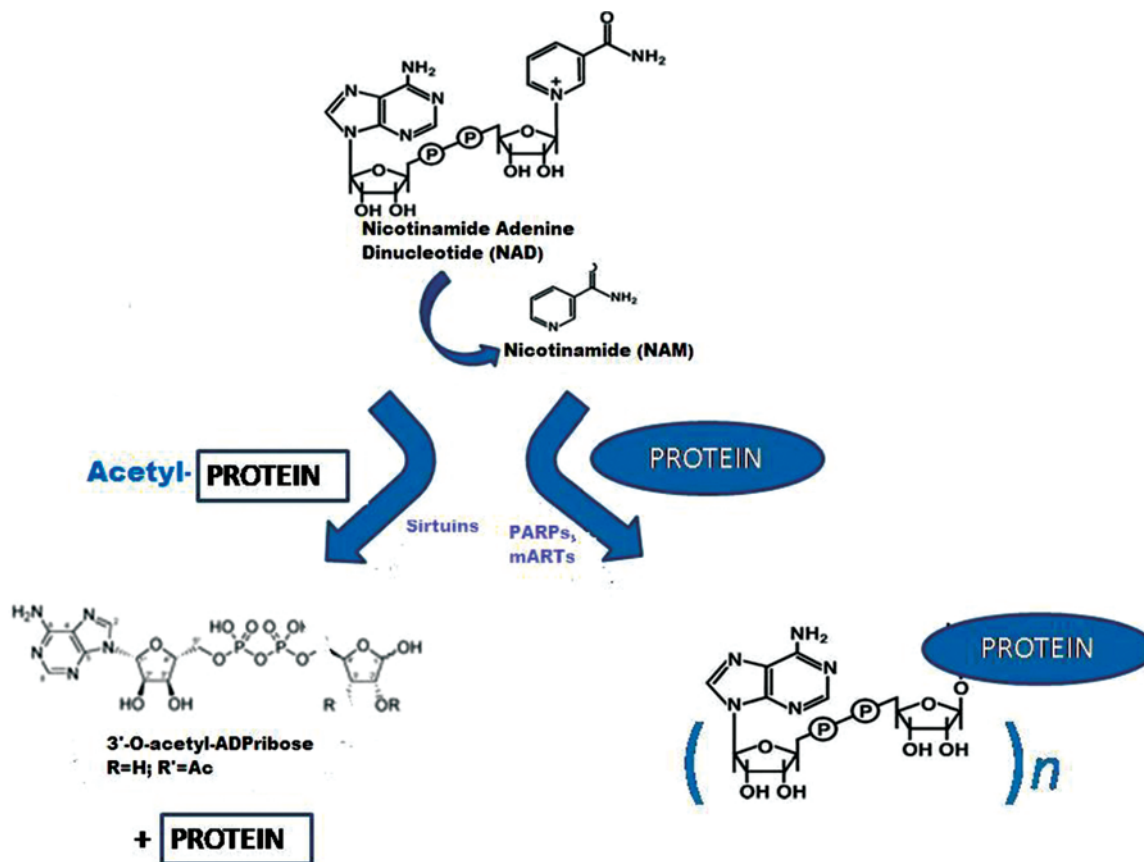


Figure 1. The reactions of NAD⁺-dependent ADP-ribose synthesis by Sirtuins and ADP-ribosyl transferases. Sirtuins transfer the acetyl group from a substrate protein to ADP-ribose. ADP-ribosyl transferases transfer ADP-ribose to an acceptor protein. mARTs, mono- ADP-ribosyl transferases; PARPs, poly- ADP-ribosyl transferases.

directly link environmental signals to mammalian stress response and metabolic homeostasis (35, 36). The fact that sirtuins require NAD⁺ for their enzymatic activity links directly the activity of sirtuins to the metabolic state in the cell and crosses metabolism with aging and aging-related diseases (cancer, neurodegeneration, cardiovascular disease) (37). Recent work suggests that sirtuins can modulate ROS levels notably during a dietary regimen known as Calorie Restriction (CR) which enhances lifespan for several organisms (38-39). The most direct indication that sirtuins play an important role in the physiological adaptation to CR comes from a more detailed analysis of their substrates and physiological effects (40, 41). The effects of CR must be a coordinated, systemic response, involving various tissues and the way they interact (41). The hallmarks of CR are metabolic adaptation to oxidative metabolism in order to produce as much as possible energy from available sources,

and resistance to stress, particularly oxidative stress (40-43). Although both sirtuins and ROS have been implicated in the aging process, their precise roles remain almost unknown.

SIRT1 plays a central role in inducing stress resistance, mitochondrial biogenesis, and fat metabolism (43, 44). This sirtuin deacetylates PGC-1alpha (45, 46), FOXO1(47, 48), and PPARalpha (49). Moreover, SIRT1 activity is tightly linked to AMP kinase (AMPK) (50, 51), since AMPK regulates expression of the NAD synthetic enzyme NAMPT (52), and SIRT1 was shown to deacetylate and to activate the AMPK activator kinase LKB1 (53) although the latter has been recently attributed to SIRT3 (54). At the same time, SIRT1 turn off glycolytic metabolism by deacetylating glycolytic enzymes (55) and one of their key transcriptional inducers, HIF-1alpha (56). The metabolic shift

from glycolysis toward mitochondria processes, and the derived stress tolerance are sustained by the activity of SIRT3 and SIRT5 that deacetylates and desuccinylates mitochondrial proteins respectively (57-59). Mitochondrial superoxide dismutase 2 and metabolic enzymes of fatty acid oxidation, the urea cycle, and acetate metabolism are main targets of these sirtuins (43, 60-66). SIRT3 reduces reactive oxygen species (ROS) production by mitochondria, thus blocking HIF-1 α induction and reducing the ROS charge to cells (67, 68). SIRT6 deacetylates histones at HIF-1 α loci corepressing HIF-1 α target (69).

As opposite to SIRT3 and SIRT5, the expression of the third mitochondrial sirtuin, SIRT4, decreases in CR. This is explainable as SIRT4 ADP-ribosylates and represses glutamate dehydrogenase (70), a key enzyme allowing glutamine and glutamate to enter Krebs' cycle and central metabolism to provide energy during CR. This co-involvement of SIRT1, SIRT3, SIRT4, and SIRT6 is relevant to the Warburg effect, in which cancer cells show a massive increase of glycolysis and glutaminolysis, leading to possible tumor suppressor functions (71). Supporting this hypothesis are the findings that in many tumors loss of SIRT3 or SIRT6 (increase of glycolysis), or loss of SIRT4, inducing glutaminolysis, have been measured (69, 71-73). Moreover, SIRT4 is mostly increased in DNA damage and blocks glutamine entry into metabolism (72). As a consequence it might be possible a tumor suppressor role by a "glutamine checkpoint" that limits growth of precancerous cells to allow repair of damage (71, 72). The involvement of SIRT4 in cancer suppression is supported by another study where the levels of this sirtuin are regulated by mTORC1, a protein complex that functions as a nutrient/energy/redox sensor and controls protein synthesis (74). Some observations about sirtuin functioning in tumors go to different direction. For instance for SIRT1, there is evidence for both a tumor prevention function (75) and tumor enhancement function in established tumors (76).

In synthesis sirtuins with the nutrient sensors cited above, (AMPK, mTOR, FOXO, etc.) are considered very important in linking diet, metabolism, aging and diseases (reviewed in 38, 40, 41). This view supports the initial hypothesis made by Guarente that nutrient-sensing regulators mediate the effects of diet on aging and diseases (28). He based it only on the findings that sirtuins were

NAD⁺-dependent protein deacetylases and known to contrast aging in yeast. At present, more than a decade later, many data from mammals suggest an elaborate set of physiological adaptations to caloric intake mediated by sirtuins. The interactions of sirtuins and all nutrient sensors are summarized in a very recent and interesting review reporting an emerging concept, the mitohormesis, explaining the way ROS not only do cause oxidative stress, but rather may function as signaling molecules that promote health by preventing or delaying a number of chronic diseases, and ultimately extend lifespan (77). Therein is discussed that while high levels of ROS are generally accepted to cause cellular damage and to promote aging, low levels of these radicals may rather improve systemic defense mechanisms by inducing an adaptive response (77).

In the light of the present knowledge, sirtuins, belonging to class III histone deacetylase family, are considered as epigenetic regulators of metabolism as well as other cellular processes and seem ideal targets of future therapeutical interventions (78, 79). The finding that SIRT1 is activated by small molecules, like resveratrol, able to alter their activities (SIRT1-activating compounds, STACs) through an allosteric mechanism (78), allows to hypothesize that small molecules that bind to the SIRT1 allosteric site might be like natural endogenous compounds that regulate the enzyme under certain physiological conditions, for instance in CR (40, 41). The synthesis of analogs of these small molecules with regulatory functions might open new frontiers for pharmacological interventions.

4. THE FAMILY OF PARPS

In the last 20 years studies on ADP-ribosylation reactions focused on two classes of NAD⁺-dependent enzymes, PARP1 and the ecto- / endo cellular mono-ADP-ribosyltransferases (mARTs). A wide and detailed literature is available on these topics (reviewed in 6-8, 80- 82). Now it is known that several enzymes defined as poly-ADP-ribose-polymerases on the basis of a highly conserved catalytic domain, may function exclusively as a family of endogenous mono-ADP-ribosyltransferases, providing new perspectives about their functions (80, 81).

Poly(ADP-ribose) polymerases (PARPs) are enzymes that transfer ADP-ribose groups to target proteins and thereby affect various nuclear and cytoplasmic processes, Fig 1 (81, 82). In eukaryotic

Table 2. Mammalian ADP-ribosyltransferases (81)

New nomenclature	Old nomenclature	Amino acid number
ARTD1	PARP1	1014
ARTD2	PARP2	583
ARTD3	PARP3	540
ARTD4	PARP4 (vault-PARP)	1724
ARTD5	PARP5 (tankyrase-1)	1327
ARTD6	PARP6 (tankyrase-2)	1166
ARTD7	PARP15 (BAL3)	776
ARTD8	PARP14 (BAL2)	1801
ARTD9	PARP9 (BAL1)	854
ARTD10	PARP10	1025
ARTD11	PARP11	334
ARTD12	PARP12	701
ARTD13	PARP13	902
ARTD14	PARP7	657
ARTD15	PARP16	322
ARTD16	PARP8	854
ARTD17	PARP17	631

cells a family of enzymes termed poly(ADP-ribose) polymerases (PARPs) occurs and in the human genome, 17 different genes have been identified encoding all PARP family members, Table 2 (8, 81). PARPs participate in regulating not only cell survival and cell death programs, but also other biological functions exhibited by novel members of the PARP family (8, 81, 82). Among such functions are transcription regulation, telomere cohesion, mitotic spindle formation during cell division, and intracellular energy metabolism (8).

The activities and functions of the other PARPs have not been studied to the same extent as PARP-1, although roles for some of the PARP family members have been emerging, discussed in more detail elsewhere (83-85).

Here we focus on the nuclear poly-ADP-ribose-polymerases (mainly PARP 1 and 2) and their product, poly-ADP-ribose (PAR). PAR is heterogeneous with respect to length (as many as 200 ADP-ribose units *in vitro*) and extent of branching (approximately one branch per 20-50 ADP-ribose units) (86). The significance of this

heterogeneity in PAR function is unknown, but it could play a role in determining specific functional outcomes *in vivo* (81, 82). PAR may alter protein activity by functioning as a site-specific covalent modification, a protein-binding support, or a steric hindrance structure (86, 87).

Poly ADP-ribose glycohydrolase (PARG) and ADP-ribosyl hydrolase 3 (ARH3) catalyze the hydrolysis of PAR producing free mono and oligo(ADP-ribose) (21-23). ADP-ribosyl protein lyase cleaves the final remaining ADP-ribose monomer from the target protein, releasing ADP-3"-deoxypentose-2"-ulose (ADP-DP) (88).

The central role of PARPs, mainly PARP- 1 and -2, is to contribute repairing DNA damage raised from excessive oxidative stress. Accumulation of DNA damage can lead to cell cycle arrest or genomic instability, occurring in the aging process. A common feature of PARP-1 and PARP-2 in DNA repair is automodification (86). PARP-1 and PARP-2 can heterodimerize and ADP-ribosylate each other, which may play a role in mediating efficient base excision DNA repair. How the heterodimerization and cross-modification occurs, as well as their exact function *in vivo*, are still largely unclear.

While PARP-1 function is known to be critical for the long-term maintenance of genomic stability through the regulation of chromatin structure, cell cycle arrest, DNA repair and apoptosis (89-91), PARP-2 was originally described in connection to DNA repair and in physiological and pathophysiological processes associated with genome maintenance (e.g., centromere and telomere protection, spermiogenesis, thymopoiesis, azoospermia, and tumorigenesis) (92-95). Recent reports have identified important rearrangements in gene expression upon the knockout of PARP-2 (96).

Recruitment of PARPs during the DNA repair process can lead to widespread metabolic changes. These results have identified novel metabolic responses in cancer cells following DNA damage and on inhibiting activity of proteins involved in DNA repair processes (97).

It is known that PARP-1 activity participates directly in necrotic or apoptotic cell death and enhances inflammatory signalling and secondary damage (98). It is widely documented that PARP-1 and PAR respond to a wide variety of signals raised from

oxidative, nitrosative, genotoxic, oncogenic, thermal, inflammatory, and metabolic stresses (99). The consequences of stress conditions are pathologies, including cancer, inflammation-related diseases, and metabolic dysregulation (98-100). From recent data the originally described DNA damage repair PARP 1 and 2, seem to play a role in metabolic regulation by influencing mitochondrial function and oxidative metabolism, with a major impact on metabolism and its alterations (101).

Key roles for PARP-1 and PARP-2 in metabolic stress and homeostasis have been proposed (101). Insufficient or excessive nutrients induce cells to rearrange their metabolism, energy stores/expenditure. Such rearrangements heavily impact inflammation. Lifestyle and dietary changes may affect modulation of PARP-1 function to improve health (101). The adaptation ability of cells towards metabolic stress drives the physiological or pathological states, as metabolic- or age-related diseases (obesity, diabetes, cancer) (102). Some of the effects of PARP-1 on adipogenesis are due to direct effects on PPARgamma-dependent adipogenic gene expression in fat cells (103). The nuclear receptors, peroxisome proliferator-activated receptors (PPARs), have well-documented roles in lipid and glucose metabolism (104).

5. THE INTERPLAY OF SIRTUINS AND PARPS

The scenario emerging from the previous sections prompted researchers to hypothesize an interplay between PARPs and sirtuins: highly conserved in eukaryotes, share NAD^+ as a common substrate, produce nicotinamide (NAM) and ADP-ribose (105).

PARP-1 is a major NAD^+ consumer in the cellular processes, in which the ADP-ribose moiety is not transferred to an acetyl group, as sirtuins do, but to acceptor proteins in order to build ADP-ribose polymers, Fig 1 (81, 82). On this basis, cellular NAD^+ levels may mediate the functional interplay between PARP-1 and SIRT1 and determine the final cell fate. Hyperactivation of PARP-1 upon severe oxidative damage causes rapid depletion of intracellular NAD^+ levels because PARP-1 uses NAD^+ as the endogenous substrate for poly-ADP-ribosylation (81, 82). PARP1 becomes activated by binding to DNA breaks and as a result the ADP-ribosylation activity of PARP1 increases 10–500 fold. Therefore, SIRT1 activity is down-regulated

during PARP-1 hyperactivation. These observations indicate that PARP-1 and SIRT1 activity are inter-dependent as they compete for the same pool of cellular NAD^+ (105). Moreover, excessive NAD^+ consumption by PARP-1 hyperactivation depletes intracellular ATP levels leading to the release of apoptosis-inducing factors (AIF) and consequent cell death due to energy reduction (105, 106). Inhibiting PARP-1, SIRT1 activity increases, perhaps through increased NAD^+ availability (105).

SIRT1 acts as an intracellular NAD^+ sensor that translates changes of the metabolic/redox state of the cell into adaptive transcriptional responses (105-107). NAD^+ levels control SIRT1 activity; they can rate-limit SIRT1, that has a $K_m^{\text{NAD}^+}$ close to the physiological concentration of NAD^+ (105). Moreover nicotinamide, one of the PARP and sirtuin reaction products, is an allosteric inhibitor of SIRT1 activity (108).

The competition of SIRT1 and PARP1 for NAD^+ is only one feature of their interaction. PARP-1 activity decreases after deacetylation by SIRT1 and enzyme expression is reduced by SIRT1 regulation of PARP-1 gene promoter (109).

One can hypothesize that a fine regulation of PARP activity through NAD^+ levels and SIRT1s may be essential to prevent the development of several age-related pathological disorders, where the oxidant-mediated cell injury is dependent on PARP activation. To this regard Bai laboratory reported that oxidative stress of cells by exposure to H_2O_2 , induces PARP-1 activation and SIRT1 inhibition, as PARP-1 highly consumes the available NAD^+ , in competition with SIRT1 (110).

On the other hand a negative transcriptional regulation of SIRT1, independent on cellular NAD^+ levels, is mediated by PARP-2, that localizes to the SIRT1 promoter (111). Depleting PARP2 SIRT1 activity increases and allows to deacetylate SIRT1 targets, as PGC-1 α , a transcriptional coactivator of nuclear encoded mitochondrial genes (110, 111). As a consequence, mitochondrial biogenesis and fat oxidation are promoted, meanwhile the onset of diet-induced obesity is counteracted (111). Thus PARPs influence SIRT1 both by limiting NAD^+ availability (PARP-1) and by regulating transcriptionally SIRT1 expression (PARP-2) (110, 111). Their actions, even different, link DNA damage levels (physiological to excessive) with mitochondrial oxidative processes and energy metabolism, and suggest strong

metabolic implications and possible physiological outcomes in response to metabolic stress.

A hypothesized mechanism regards the Warburg effect, the anaerobic glycolysis, a key process for malignant transformation. PARP inhibitors have been proposed as anti-Warburg agents stimulating oxidative metabolism (112). Being able to act on both PARP-1 and PARP-2, they might contribute to potentiate SIRT1 function (depending on PARP-1 inhibition and increase of NAD^+ levels), and to regulate the transcriptional induction of SIRT1 expression following PARP-2 inhibition. This mechanism might work also in impaired oxidative processes occurring in mitochondrial diseases.

An interesting finding opposite to the transcriptional inhibition of SIRT1 expression regards PARP-2 as a transcriptional enhancer of PPARGgamma. PPARs act as lipid sensors, being differently stimulated by lipid species (104), and themselves control SIRT1 activity. PPARGgamma is related to lipid anabolism, inhibiting SIRT1 expression, while PPARalpha and PPARbeta/delta, both linked to fatty acid oxidation, increase SIRT1 mRNA levels. By considering that PPARGgamma is regulated by PARP-1 too, and that PARP-2 deletion/inhibition leads to SIRT1 activation, enhancing oxidative metabolism and protection against diet-induced obesity and insulin resistance (113, 114), the link PARP, sirtuins, metabolism is evident. Interestingly, PARP inhibition enhances the activity of SIRT1, but not that of SIRT2 or SIRT3. The major difference between these three sirtuins is their subcellular localization, as, amongst them, only SIRT1 is a nuclear sirtuin. This suggests the existence of compartment-specific NAD^+ pools in the cell. Supporting this possibility, elegant studies by the Sinclair lab showed the existence of independently regulated NAD^+ pools (115).

SIRT1 and PARP1 play also a common role responding to DNA damage and the lack of either of these proteins may lead to DNA damage sensitization. Novel interconnections between DNA repair, metabolism, and circadian rhythms have been proposed and seem to involve both SIRT1 and PARP1 (116, 117). The core circadian complex involves a transactivating CLOCK/BMAL1 heterodimer, which induces the transcription of a large number of genes, including the cryptochrome (CRY1 and CRY2) and period (PER1, PER2, PER3) genes that form a complex that leads to a negative feedback loop suppressing CLOCK/

BMAL1-mediated transcription. Several studies have shown that alteration of core circadian interactions can lead to dysregulation of DNA damage repair (116, 117). Despite it is not known yet whether a regulatory link exists between SIRT1 or PARP1 and the circadian components during DNA damage, the interaction between CRY and BMAL1 is destabilized upon deacetylation of BMAL1 at K537 by SIRT. Moreover CLOCK possesses acetyltransferase activity that regulates the transcriptional activity of CLOCK/BMAL1 and is capable of acetylating some of the components that SIRT1 deacetylates (117, 118). At last, it has been shown also that PARP1 has rhythmic activity influenced by feeding patterns. PARP1 is capable of ADP-ribosylating CLOCK in a circadian manner disrupting the association between the BMAL1/CLOCK heterodimer and its targets (A66) (117, 118).

Another sirtuin family member involved in DNA repair is SIRT6 that possesses both deacetylase and mono(ADP-ribosyl) transferase activities (119, 120). There is evidence that SIRT6 overlaps functions of PARP-1 in stress signaling, including roles in genome stability, NF-kappaB-mediated stress signaling, and metabolism (121). SIRT6 recognizes double-strand break sites in DNA upon oxidative stress conditions. It mono(ADP-ribosyl)ates PARP-1 inducing its activation. PARP-1-dependent DNA repair pathways starts thereafter, further enhancing double-strand break repair under oxidative stress (121).

6. CONCLUDING REMARKS

The mammalian sirtuins, as well as the main members of PARP family, have been connected to various activities from cellular stress resistance and genomic stability, to tumorigenesis and energy metabolism (9, 10, 122).

Among the members of the two protein families discussed here the better studied members are SIRT1 and PARP1/2. The modulation of NAD^+ levels through the competition of PARP1 and SIRT1 for the pyridinic dinucleotide seems to be a promising strategy to control SIRT1 activity in order to achieve health benefits.

SIRT1 activity has been postulated as a mediator of the beneficial effects of calorie restriction on health- and life-span. As a mediator of the metabolic and transcriptional adaptations to situations of energy stress and nutrient deprivation,

SIRT1 activation enhances fat consumption and uses mitochondrial respiration to implement energy harvesting.

Metabolic disease has been strongly linked to impaired energy homeostasis and mitochondrial function. The interaction between PARP-1 and SIRT1 activities supports the known involvement of PARPs in ageing. A recent literature reports that PARP activity is higher in aged tissues, leading to decreased SIRT1 activity (123). Although the regulation of SIRT1 and PARP1 is controlled by a variety of metabolic, genotoxic and circadian stimuli, scarce is the knowledge how these stimuli affects the regulatory network of both proteins. For instance about circadian regulation it is still unknown the influence that DNA damage response may have and the roles played by SIRT1 and PARP1 (117, 118).

PARP inhibition could be a nice strategy to activate SIRT1 and mimic the calorie-restricted state. Both SIRT1 and PARP1 are involved in the regulation of genomic stability, and open new perspectives in studying modulators of SIRT1 and PARP1 activity as therapeutics for cancer and metabolic disorders. The metabolic functions of PARP-1 and PARP-2, in concert with SIRT1, suggests a therapeutic potential of PARP inhibitors in such disorders (112).

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