Protein family review

**Sirtuins: Sir2-related NAD-dependent protein deacetylases**
Brian J North and Eric Verdin

Address: Gladstone Institute of Virology and Immunology, University of California, San Francisco, CA 94141, USA.

Correspondence: Eric Verdin. E-mail: everdin@gladstone.ucsf.edu

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**Summary**

Silent information regulator 2 (Sir2) proteins, or sirtuins, are protein deacetylases dependent on nicotine adenine dinucleotide (NAD) and are found in organisms ranging from bacteria to humans. In eukaryotes, sirtuins regulate transcriptional repression, recombination, the cell-division cycle, microtubule organization, and cellular responses to DNA-damaging agents. Sirtuins have also been implicated in regulating the molecular mechanisms of aging. The Sir2 catalytic domain, which is shared among all sirtuins, consists of two distinct domains that bind NAD and the acetyl-lysine substrate, respectively. In addition to the catalytic domain, eukaryotic sirtuins contain variable amino- and carboxy-terminal extensions that regulate their subcellular localizations and catalytic activity.

**Gene organization and evolutionary history**

Protein acetylation regulates a wide variety of cellular functions, including the recognition of DNA by proteins, protein–protein interactions, and protein stability (reviewed in [1]). Post-translational modification of proteins at lysine residues by reversible acetylation is catalyzed by the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs), which act on both histone and non-histone substrates despite their names. HDACs are grouped into three classes on the basis of their homology to yeast transcriptional repressors. Class I and class II HDACs, which share significant similarity to each other in their catalytic cores, are homologs of the yeast deacetylases Rpd3p and Hda1p, respectively (reviewed in [2,3]). The class III HDACs are homologous to the yeast transcriptional repressor Sir2p and have no sequence similarity to class I and II HDACs; these Sir2 proteins, also called sirtuins, are the focus of this article.

The founding member of class III HDACs, *Saccharomyces cerevisiae* Sir2p, functions in transcriptional repression at the telomeres [4,5], the silent mating-type loci [6-8], and ribosomal DNA loci [9,10]. Sir2p has been implicated in the repair of double-strand DNA breaks, cell-cycle progression, and chromosomal stability in yeast and plays a pivotal role in the molecular mechanisms of aging in both *S. cerevisiae* and *Caenorhabditis elegans* [11-15].

Sirtuins have been found in bacteria to eukaryotes [16,17]. The hallmark of the family is a domain of approximately 260 amino acids that has a high degree of sequence similarity in all sirtuins. The family is divided into five classes (I-IV and U) on the basis of a phylogenetic analysis of 60 sirtuins from a wide array of organisms [17] (Figure 1). Class I and class IV are further divided into three and two subgroups, respectively. The U-class sirtuins are found only in Gram-positive bacteria [17]. The *S. cerevisiae* genome encodes five sirtuins, Sir2p and four additional proteins termed ‘homologs of sir two’ (Hst1p-Hst4p) [11] (Figure 1). The human genome encodes seven sirtuins, with representatives from classes I-IV (Table 1) [17].

**Characteristic structural features**

The sirtuins have a catalytic domain, unique to this family, characterized by its requirement for nicotine adenine dinucleotide (NAD) as a cofactor [18]. The structures of four sirtuins (Archaeoglobus fulgidus Sir2-Af1 and Sir2-Af2, human...
Sirt2, and yeast Hst2) have been obtained at atomic resolution, and a number of common features are emerging [18-21]. All four are organized in two bilobed globular domains: a small domain with two distinct modules and a large domain (Figure 2).

**The large domain**

The large domain contains an inverted classical open α/β Rossmann-fold structure, which is commonly found in proteins that bind oxidized or reduced NAD or NADP [18,20,21] (Figure 2). This domain consists of six parallel β strands (β1-β3 and β7-β9) that form a central β sheet, and eight α helices (α1, α2, α6, α7, and α9-α12) that pack against the β sheet (Figure 2). Sirt2 and the yeast Hst2p each have an additional α helix in the crystal structure, α13, that packs against the outside of the large domain but is not found in Sir2-Af1 or Sir2-Af2. The most significant difference in the large domains is an insertion in the region of the α11 helix.
enzymatic activity [20]. The localization of zinc away from the NAD-binding pocket suggests that the zinc ion does not participate directly in catalysis. This is in contrast to class I and class II HDACs, in which zinc ions are part of the active site [20,23].

The NAD-binding pocket

The NAD-binding pocket, located within the large domain at the interface of the large and small domains, can be divided into three spatially distinct regions: the A site, where the adenine-ribose moiety of NAD is bound, the B site where the nicotinamide-ribose moiety is bound, and the C site, located deep in the NAD-binding pocket (see also Figure 3) [20]. The B and C sites are thought to be directly involved in catalysis. In the presence of an acetyl-lysine, NAD bound to the B site can undergo a conformational change, bringing the nicotinamide group in proximity to the C site, where it can be cleaved [20]. The ADP-ribose product of this reaction may then return to the B site, where deacetylation of the acetyl-lysine occurs. The organization of the NAD-binding pocket might explain how nicotinamide inhibits sirtuin activity. At high concentrations, free nicotinamide may occupy the C site, irrespective of whether any acetylated lysine is bound, and block the conformational change of NAD [24].

The large groove

A large groove is formed at the interface between the large and small domains and runs perpendicular to the long axis formed by the two domains (Figure 2). On the basis of mutagenesis studies, a role in substrate recognition and catalysis has been proposed for this groove [18,20]. Analysis of the crystal structure of A. fulgidus Sir2-Af2 complexed with a p53 peptide (Figure 2b) demonstrates that the peptide substrate lies in the large groove [21]. The binding of an acetylated peptide, such as p53, may occur through the formation of an enzyme-substrate β sheet, in which the substrate β strand is sandwiched between the β11 strand within the Rossmann fold and a β10 strand within the FGE loop, named for its highly conserved FGE XL motif [21]. A high degree of conservation between sirtuins is found in the residues implicated in substrate peptide binding, specifically in the region of the FGE loop [21]. The predicted β10 and β11 strands of Sir2-Af2 are absent from the solved structures of Sirt2, Sir2-Af1, and Hst2p, however. These observations suggest that these two regions become more ordered and form the enzyme-substrate β sheet upon substrate binding, as seen in the Sir2-Af2 p53 structure [21].

Amino- and carboxy-terminal extensions

The solved structures of full-length A. fulgidus Sir2-Af1, A. fulgidus Sir2-Af2 and human Sirt2 correspond primarily to the catalytic domain found within sirtuins. Yeast Hst2 and human Sirt2 have large amino- and carboxy-terminal extensions (not shown for Sirt2 in Figure 2c) that are likely to play a role in the regulation of enzymatic activity. In support of

Table I

| Name | Class | Chromosomal location | OMIM ID | Orthologs |
|------|-------|----------------------|---------|-----------|
| Sirt1 | Ia    | 10q21.3              | 604479  | Sirt2, Hst1, Sirt2β |
| Sirt2 | Ib    | 19q13.2              | 604480  | Hst2, Sirt2α |
| Sirt3 | IIb   | 11p15.5              | 604481  | Hst2, Sirt2α |
| Sirt4 | II    | 12q24.31             | 604482  | Sirt4      |
| Sirt5 | III   | 6p23                 | 604483  | Sirt5      |
| Sirt6 | IVa   | 19p13.3              | 606211  | Sirt6      |
| Sirt7 | IVb   | 17q25.3              | 606212  | Sirt7      |

Mouse orthologs of human Sirt4-7 have not been characterized but are found in sequence databases. OMIM IDs are from the Online Mendelian Inheritance in Man repository at the National Center for Biotechnology Information (NCBI) [76].
Figure 2
Three-dimensional high-resolution crystal structures of four sirtuin proteins. The zinc-binding module is shown at the top left in each panel, the helical modules of the small domain are lighter and at the top right, and the large Rossmann-fold domain is in the lower half. Each α helix and β strand is labeled to facilitate comparisons. (a) Sir2-Afl1 complexed with NAD (in stick representation; PDB accession number: 1ICI) [20]. (b) Sir2-Afl2 complexed with acetylated p53 peptide (in stick notation, with acetyl-lysine darker). Two β strands (β10 and β11) are shown that might mediate the binding of the substrate peptide (PDB accession number: IMA3) [21]. An acetylated peptide, such as p53, may be bound through the formation of an enzyme-substrate sheet, in which the substrate strand is sandwiched between the β11 strand within the Rossmann fold and a β10 strand within the FGE loop, named for its highly conserved FGEExL motif [21]. (c) Human Sirt2 (catalytic core; PDB accession number: 1J8F) [18]. (d) Full-length yeast Hst2p with the carboxy-terminal α14 helix interacting with the NAD-binding pocket (PDB accession number: 1Q14) [19]. Structural coordinates were taken from the Protein Data Bank and models were drawn with PYMOL [77].
Hst2p indicates that the carboxy-terminal extension folds into the NAD-binding pocket and that the amino-terminal extension may occlude the substrate binding cleft [19]. The carboxy-terminal α14 helix of yeast Hst2p interacts extensively with residues within the large groove between the large and small catalytic domains of the protein, as well as with residues in these domains. In the apo (unbound) form of Hst2p the α14 helix probably partially occludes the cofactor NAD-binding site and the loop following β1 is disordered. NAD binding then promotes dissociation of the α14 helix and ordering of the loop after β1, to facilitate enzymatic activity. Likewise, the human Hst2p homolog Sirt3 is enzymatically inactive as a full-length protein and becomes catalytically active after proteolytic cleavage of its amino terminus following import in the mitochondrial matrix [25].

**Localization and function**

Sirtuin proteins have been found in a wide variety of subcellular locations. Human Sirt1 localizes to the nucleus, as does yeast Sir2p [26]; Sirt1 appears to repress transcription in the nucleus by various different mechanisms. Human Sirt2 and Sirt3 localize to extra-nuclear compartments, much like yeast Hst2p: Sirt2 is found in the cytosol [27,28], whereas Sirt3 is found primarily in the mitochondria [25,29] (see Figure 4).

A key function of sirtuins is their regulation of transcriptional repression, mediated through binding of a complex containing sirtuins and other proteins. The silencing function of the yeast Sir2p is mediated by different multiprotein complexes at different genomic sites. Silencing at the telomerases and at the mating-type loci is mediated by a Sir2p complex consisting of Sir2p and the structurally unrelated proteins Sir3p and Sir4p. Sequence-specific DNA-binding proteins mediate the initial recruitment of Sir protein complexes to the telomerases and mating-type loci. At those loci, Sir3p and Sir4p blanket the underlying nucleosomes by interacting with hypoacetylated tails of histones H3 and H4, and Sir2p interacts with Sir3p and Sir4p (reviewed in [30]). Sir2p-dependent silencing of ribosomal DNA (rDNA) is mediated by the ‘regulator of nucleolar silencing and telophase exit’ (RENT) complex, containing Sir2p, Net1p, and a telophase-regulating phosphatase, Cdc14p, which is released in late metaphase [31,32]. At rDNA loci, Sir2p can silence transcription of RNA polymerase II-dependent marker genes inserted within the rDNA array and also suppresses homologous recombination among the tandemly repeated rDNA copies [9,33,34].

**Enzyme mechanism**

Initial enzymatic experiments with sirtuins, carried out in the bacterium *Salmonella typhimurium*, revealed only their activity as ADP-ribosyltransferases, not protein deacetylases. During the biosynthesis of cobalamin (also known as vitamin B12), the CoT enzyme in *Salmonella* catalyzes the transfer of phosphoribose from nicotinic acid...
mononucleotide (NaMN) to dimethylbenzimidazole (DMB) to form DMB-5'-ribosyl-phosphate [35,36]. In the absence of CobT, the Sir2-like protein CobB can partially compensate in the catalysis of this reaction [37]. In later studies, recombinant Sir2 proteins from both bacteria and humans were shown to have NAD-dependent ADP-ribosyltransferase activity in vitro [16,38]. Although the enzymatic function initially associated with sirtuins was ADP-ribosyltransferase activity, NAD-dependent histone-deacetylase activity was subsequently shown to be the primary enzymatic activity of Sir2p and other sirtuins [39-41]. The deacetylation reaction generates three products: acetyl-ADP-ribose, nicotinamide, and a deacetylated peptide substrate (Figure 3). The ratio of these products is 1:1:1, consistent with the model that hydrolysis of one NAD to acetyl-ADP-ribose and nicotinamide occurs for each acetyl group removed, and that deacetylation requires an enzyme-ADP-ribose intermediate (Figure 3) [42]. The demonstration that the ribosyltransferase and NAD-cleavage activities are both dependent on an acetylated substrate confirms the fundamental link between the two activities [38,39,42-44]. The recent observation that O-acetyl-ADP-ribose delays oocyte maturation and cell division in blastomere-stage embryos suggests that this compound might be a bona fide second messenger linked to the enzymatic activity of sirtuins [43-45]. It remains likely that there is a physiological role for the ADP-ribosyltransferase activity of individual sirtuins. A recently identified Trypanosoma brucei class Ib sirtuin, TbSIR2RP1, exerts both histone-deacetylase and robust ADP-ribosyltransferase activity on histones H2A and H2B [46]. García-Salcedo et al. [46] have suggested that the activity of TbSIR2RP1 and extent of chromatin ADP

Figure 4
Localization and functions of the human sirtuins Sirt1, Sirt2 and Sirt3. Sirt1 is found in the nucleus; it associates with several partners and targets for deacetylation, as discussed in the text. Sirt2 is found in the cytoplasm bound to the microtubule network, where it forms a complex with the histone deacetylase HDAC6. Both proteins can deacetylate α-tubulin. Sirt3 is imported into the mitochondrial matrix and proteolytically processed by the peptidase MPP, leading to its enzymatic activation (asterisk). Ac, acetyl moiety; ProteinX, an unknown protein substrate.
ribosylation correlates with the sensitivity of trypanosomes to agents that damage DNA.

It is not clear whether all five classes of sirtuins have deacetylase activity. For instance, only class I human sirtuins (Sirt1, Sirt2, and Sirt3) have robust enzymatic activity on a peptide corresponding to the amino-terminal tail of histone H4 [47]. Sirt5, a class III sirtuin, has low but detectable activity in comparison with the class I sirtuins [47]. Interestingly, the class III sirtuin from A. fulgidus, Sir2-Af1, also has low activity on a histone peptide but significantly stronger activity on an acetylated bovine serum albumin substrate, suggesting that the level of deacetylase activity is substrate-specific [20,21].

Phenotypic screens using yeast strains that have either URA3 or TRP1 inserted within Sir2p-silenced loci have led to the identification of α-substituted β-naphthol compounds, sirtinol and splitomicin, which inhibit the enzymatic activity of Sir2 proteins [48,49]. Sirtinol inhibits recombinant Sir2p and human Sirt2 in vitro and silencing at the telomeres, silent mating-type, and rDNA loci in vivo. Sirtinol was recently used to define further the role of mammalian Sirt2 proteins in the regulation of muscle gene expression and differentiation in response to alterations in the ratio of concentrations of NAD⁺ and NADH [50].

Other inhibitors have been developed that capitalize on the dependence of the sirtuins’ enzymatic activity on NAD. A nonhydrolyzable NAD molecule, carba-NAD, inhibits sirtuin activity, consistent with the requirement for NAD cleavage during the enzymatic reaction [42]. It is unlikely, however, that this inhibitor will prove useful in vivo because it cannot permeate cells and because of its potential to affect other cellular NAD-dependent enzymatic activities or biological pathways [48]. Nicotinamide, a byproduct of the NAD-dependent deacetylation reaction, inhibits sirtuins both in vitro and in vivo [42,51,52].

A recent study found a number of compounds that increase the enzymatic activity of sirtuins [53], one of which, resveratrol, activates both yeast Sir2p and human Sirt1 in vitro and in vivo. Plant polyphenols such as resveratrol - which is found in grapes and red wine - have been associated with health benefits such as cardioprotection, neuroprotection, and cancer suppression [54-56]. Sirtuins have been implicated in the regulation of cellular and organismal aging in several model organisms (see Frontiers); regulation of sirtuins by polyphenols may provide a functional link between the effects of plant products such as resveratrol on health and longevity and the regulation of aging [53].

Substrates and functions
The functions and substrates have been studied most for human Sirt1, Sirt2 and Sirt3 and their closest yeast homologs Sir2, Hst1 and Hst2; little is known about the functions of Sirt4, Sirt5, Sirt6, and Sirt7.

Sirt1
TAF,68, a transcription factor necessary for regulation of the RNA polymerase I transcriptional complex, was the first substrate to be identified for Sirt1 [57]; the fact that S. cerevisiae Sir2p also regulates rDNA suggests that Sir2p and Sirt1 may have similar functions. Deacetylation of TAF,68 by Sir2α - the mouse ortholog of human Sirt1 - inhibits transcriptional initiation in vitro [57]. Sirt1 is reported to associate physically with the human basic helix-loop-helix (bHLH) repressor proteins hHES1 and hHEY2 [58] (Figure 4); a similar interaction is also found in Drosophila, in which the bHLH repressor proteins Hairy and Deadpan recruit a sirtuin protein [59]. The Hairy-related bHLH proteins function as transcriptional repressors and play important roles in diverse aspects of metazoan development. Sirt1 has also been shown to form a complex with the histone acetyltransferase PCAF and the muscle transcription factor MyoD, and it deacetylates both proteins [50]. Although transcriptional regulation by Sirt1 through TAF,68, bHLH, MyoD and PCAF, and p53 (see below) identify non-histone targets for the sirtuins, Sirt1 might also regulate histone acetylation directly through its interaction with sequence-specific DNA binding factors, such as COUP-TF-interacting proteins 1 and 2 (CTIP1 and CTIP2) or a MyoD/PCAF complex [59,60]. These findings implicate Sirt1 as a transcriptional repressor that functions through deacetylation of histones and non-histone proteins.

Sirt1 deacetylase activity has also been implicated in the repair of DNA damage, through its ability to deacetylate the tumor suppressor p53 [26,52], a sequence-specific transcription factor that regulates processes such as the cell cycle, cell death, and DNA repair, in response to a variety of stress signals. The p53 protein is acetylated at two lysine residues, Lys320 and Lys382, in response to DNA damage, through its ability to deacetylate the p53 tumor suppressor p53 [26,52], a sequence-specific transcription factor that regulates processes such as the cell cycle, cell death, and DNA repair, in response to a variety of stress signals. The p53 protein is acetylated at two lysine residues, Lys320 and Lys382, in response to DNA damage, leading to its activation [61-63]. Acetylation by the acetyltransferase p300 positively regulates p53 activity, and deacetylation by HDAC1 and Sirt1 negatively regulates its activity [26,63]. Sirt1-mediated deacetylation of p53 suppresses the induction of apoptosis and prolongs cellular survival in response to DNA damage [52]. Both Sirt1 and p53 can be localized in promyelocytic leukemia (PML) bodies [51], subnuclear structures that are altered or disrupted in certain tumors and in response to various different cellular stresses [64]. These studies [26,51,52,61-63] suggest that Sirt1 deacetylates the p53 tumor suppressor protein to dampen apoptotic and cellular senescence pathways.

Two studies [65,66] have used gene-targeted mutagenesis experiments in mice to examine the consequences of expressing a mutant Sir2 protein lacking part of the catalytic domain or of deleting the ortholog of the Sirt1 gene completely. McBurney et al. [65] showed that animals homozygous for a null allele of sir2α are born at only half the expected frequency, suggesting prenatal lethality; homozygous embryos and pups are smaller than their wild-type and
heterozygous littersmates and have developmental defects of the eyes, lungs, and pancreas. In an outbred background, the Sir2α-null animals often survive to adulthood, but both sexes are sterile because of a failure to ovulate in females and inefficient spermatogenesis in males [65]. Unexpectedly, there is no defect in gene silencing in Sir2α-null animals, nor in Sir2α-null homozygous embryonic stem cells, suggesting either that Sir1 orthologs have a different role in mammals from that in S. cerevisiae or that its role in gene silencing is confined to a small subset of mammalian genes [65,67]. In the second study [66], mice were generated that lacked Sir2α or expressed a mutant Sir2α protein lacking part of the catalytic domain. Both types of Sir2α mutant mice were smaller than their wild-type and heterozygous littersmates and had developmental defects in the retina and heart; most died postnatally [66]. Sir2α-deficient thymocytes showed an increase in p53 hyperacetylation in response to ionizing radiation, followed by apoptosis [66]. The phenotype of the Sir2α-null mice suggests that the Sir1 protein is essential for normal embryogenesis and for normal reproduction in both sexes in mammals. Further study of the function of Sir1 orthologs at the molecular level will help to unravel the origins of these developmental defects.

Sirt2

The human Sirt2 protein is similar in sequence to yeast Hst2p and both proteins are located in the cytoplasm [27,28] (Figure 4), where Hst2p affects chromatin silencing through an unknown mechanism [27]. Sirt2 colocalizes with the microtubule network and deacetylates Lys40 of α-tubulin [47]. The same residue of α-tubulin is also deacetylated by HDAC6, a class II HDAC, and deacetylation by HDAC6 leads to changes in cellular motility [68]. Sirt2 and HDAC6 are found along microtubules and can be co-immunoprecipitated with each other, suggesting that the two proteins coordinate the level of tubulin acetylation [47]. Recent evidence shows that Sirt2 is upregulated before mitosis and suggests a role for this protein in cell-cycle regulation [69]. Sirt2 is rapidly degraded after mitosis, and cells overexpressing mutant forms of Sirt2 show a delay in exit from mitosis. Whether Sirt2-dependent regulation of α-tubulin acetylation is related to cell-cycle arrest remains to be determined.

A role for Sirt2 in cancer pathogenesis was recently demonstrated using a proteomic approach [70]. The Sirt2 gene, which is located at chromosome 19q13.2, lies within a region that is frequently deleted in human gliomas, and levels of Sirt2 mRNA and Sirt2 protein expression are severely reduced in a large fraction of human glioma cell lines [70]. Ectopic expression of Sirt2 in these cell lines suppressed colony formation and modified the microtubule network. These results indicate that Sirt2 may act as a tumor suppressor and may function to control the cell cycle by acetylation of α-tubulin.

Sirt3

Human Sirt3 is primarily located in the mitochondrial matrix [25,29] (Figure 4), and its mitochondrial import is mediated by an amphiphatic α helix at its amino terminus. The protein, whether endogenous or overexpressed, is proteolytically clipped at its amino terminus in vitro, resulting in the removal of the first 100 amino acids. This clipping can be recapitulated in vitro with purified mitochondrial-matrix processing peptidase (MPP) [25]. Interestingly, the unprocessed protein is enzymatically inactive in vitro and becomes enzymatically active after proteolytic processing by MPP [25]. Negative regulation of the enzymatic activity of a sirtuin by its amino terminus is also observed in the case of another class Ib sirtuin, yeast Hst2p. This protein may form a homotrimer in solution, such that the amino-terminal methionine of each molecule interacts with the active site in another Hst2p molecule within the quaternary structure [19].

Although Sirt3 shows robust histone deacetylase activity on a histone H4 peptide in vitro, the absence of histones in mitochondria suggest that non-histone proteins are its primary target [25,29]. Mouse Sirt3, also called Sir213, lacks the amino-terminal extension that mediates the mitochondrial targeting of human Sirt3 and is located in cytoplasmic vesicles [71]. Finally, a small fraction of cells overexpressing Sirt3 shows reproducible nuclear staining (B.J.N. and E.V., unpublished observations); the relevance of this observation is unclear, but it could indicate selective targeting of Sirt3 to different compartments under different physiological conditions.

Frontiers

Much excitement has been generated by the recent observations that sirtuin proteins might play a significant role in the genetic control of aging. In S. cerevisiae, lifespan is shortened by a null mutation in SIR2 and is extended by the presence of an extra copy of SIR2 [12,72]. Loss of Sir2p leads to a derepression of silencing at the rDNA locus, which increases recombination between rDNA repeats and results in the accumulation of extrachromosomal rDNA circles. High numbers of these circles in older mother cells promote senescence by an undefined mechanism, possibly through the titration of necessary factors away from other promoters [73]. Likewise, the C. elegans Sir2p homolog, Sir-2.1, mediates dauer-larva formation and regulates lifespan [15]. The dauer larva represents a specialized survival form of the worm; the molecular mechanisms by which Sir-2.1 controls dauer formation remain to be elucidated, however.

It has been speculated that the metabolic rate of the cell may be important in the regulation of the function of various sirtuins, given their dependency on NAD for enzymatic activity. This idea is further supported by evidence that NAD metabolism directly participates in controlling the aging process [12,74,75]. Translating these ideas to mouse and human sirtuins could give novel insights into the
regulation of mammalian lifespan. In mammals, Sirt1 could be a key part of the decision of a cell whether to live or die in response to DNA damage. As the cell ages and NAD levels become lower, the resulting reduced activity of Sirt1 could drive the decision to cell senescence or apoptosis rather than cellular survival. Likewise, lowered activity of Sirt2 via reduced levels of NAD could alter the rate of cell division through cell-cycle regulation. The localization pattern of Sirt3 in the mitochondrial matrix and its dependence on NAD suggest a possible function in the regulation of cellular metabolism as a sensor for intramitochondrial NAD levels. Further work is required to determine the endogenous target of this deacetylase in the mitochondrial compartment, however. Genetic deletion of the genes encoding different sirtuins in mice will facilitate studies of their roles in mammalian aging.

In addition to a connection between metabolism and sirtuin activity via NAD, the formation of O-acetyl-ADP-ribose as an enzymatic byproduct represents another promising area of investigation. Microinjection of O-acetyl-ADP-ribose delays or blocks oocyte maturation and cell division in blastomeres [45]. A similar effect is observed after microinjection of low levels of active yeast Hst2 or human Sirt2 enzyme, but not with a catalytically impaired mutant, indicating that the enzymatic activity is essential for the observed effects.

Human Sirt1, Sirt4, Sirt5, Sirt6, and Sirt7 show low or undetectable enzymatic activity on histone H4 peptide [47]. This could reflect differing substrate specificities or different requirements for cofactors, a field of investigation that is likely to yield interesting insights in the future. Finally, an increased understanding of the relationship between the structure and function of sirtuin proteins will be important in designing specific inhibitors and exploring their potential therapeutic value in a variety of pathological conditions.

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