# THE SIR2 FAMILY OF PROTEIN DEACETYLASES

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**Key Words**  Sir2, deacetylase, NAD$^+$, caloric restriction, aging

■ **Abstract**  The yeast SIR protein complex has been implicated in transcription
silencing and suppression of recombination. The Sir complex represses transcription
at telomeres, mating-type loci, and ribosomal DNA. Unlike *SIR3* and *SIR4*, the *SIR2*
gene is highly conserved in organisms ranging from archaea to humans. Interestingly,
Sir2 is active as an NAD$^+$-dependent deacetylase, which is broadly conserved from
bacteria to higher eukaryotes. In this review, we discuss the role of NAD$^+$, the
unusual products of the deacetylation reaction, the Sir2 structure, and the Sir2
chemical inhibitors and activators that were recently identified. We summarize the
current knowledge of the Sir2 homologs from different organisms, and finally we
discuss the role of Sir2 in caloric restriction and aging.

## CONTENTS

## THE DISCOVERY AND IDENTIFICATION OF SIR2

Studies in yeast have led to the identification of cellular factors that are required
for transcriptional silencing. Among these are the proteins encoded by the yeast
SIR genes, which are responsible for silencing at repeated DNA sequences in

yeast: mating-type loci, telomeres, and rDNA. *SIR2*, *SIR3*, and *SIR4* genes are required for silencing at mating-type loci (1) and telomeres (2), whereas the *SIR2*, but not the *SIR3* and the *SIR4*, is required for silencing in the rDNA (3, 4). Silencing causes a closed, inaccessible regional chromatin structure, as assayed by various probes of DNA accessibility (5, 6). Silencing requires particular lysine residues in the amino-terminal tail of histones H3 and H4 (7–9). These and other lysine residues of the tail are acetylated in active chromatin but deacetylated in silenced chromatin (9, 10). The deacetylated histones can fold into a more compact, closed nucleosomal structure (11). Due to global deacetylation of yeast histones observed when the SIR2 was overexpressed (10), Sir2 was suggested to be a histone deacetylase. However, early attempts to demonstrate histone deacetylase activity for Sir2 were not successful.

Unlike the *SIR3* or *SIR4*, the *SIR2* gene is highly conserved in the organisms ranging from archaea to humans (12). Studies on a bacterial homolog, *cobB*, led to the conclusion that this gene could substitute for another bacterial gene, *cobT*, in the pathway of cobalamin synthesis (13). The *cobT* encodes an enzyme that transfers a ribose-phosphate moiety from nicotinic acid mononucleotide (NMN) to dimethyl benzimidazole. Thus, it seemed possible that Sir2 proteins might catalyze a similar but different reaction because the cobalamin pathways are not present in yeast or mammals. Frye (14) showed that Sir2 proteins from bacteria, yeast, and mammals were able to transfer an ADP-ribose group from $NAD^+$ to a protein carrier. Subsequent work by others proved that the Sir2 protein was indeed an ADP-ribosyltransferase in vitro, and its activity was essential for silencing in vivo (15). Imai et al. (16) discovered that the amino-terminal tails of histone H3 or H4 peptides could accept ADP-ribose from $NAD^+$, but only if the peptides were acetylated. Through mass spectrometry, they showed that the relative molecular weight of the product was actually smaller than that of the substrate by 42 Da, indicating that the major modification catalyzed by Sir2 was deacetylation and not ADP-ribosylation. When $NAD^+$ was omitted from the reaction, Sir2 exhibited no deacetylase activity. NADH, NADP, and NADPH could not substitute for $NAD^+$ in this reaction. Landry et al. (17) characterized the role of SIR2 and HST2 in a nicotinamide-$NAD^+$ exchange reaction. HST2 had $NAD^+$-dependent deacetylase activity (17). Smith et al. (18) also characterized SIR2 and HST genes as an $NAD^+$-dependent deacetylase. They also first showed that mutation, which lowered $NAD^+$ synthesis in vivo, compromised the silencing activity of SIR2p.

## CATALYTIC MECHANISM

The Sir2-dependent deacetylation reaction is different from other HDACs. Here we review the role of $NAD^+$ and the unusual reaction products. Finally, we discuss the possible biological role of those products.
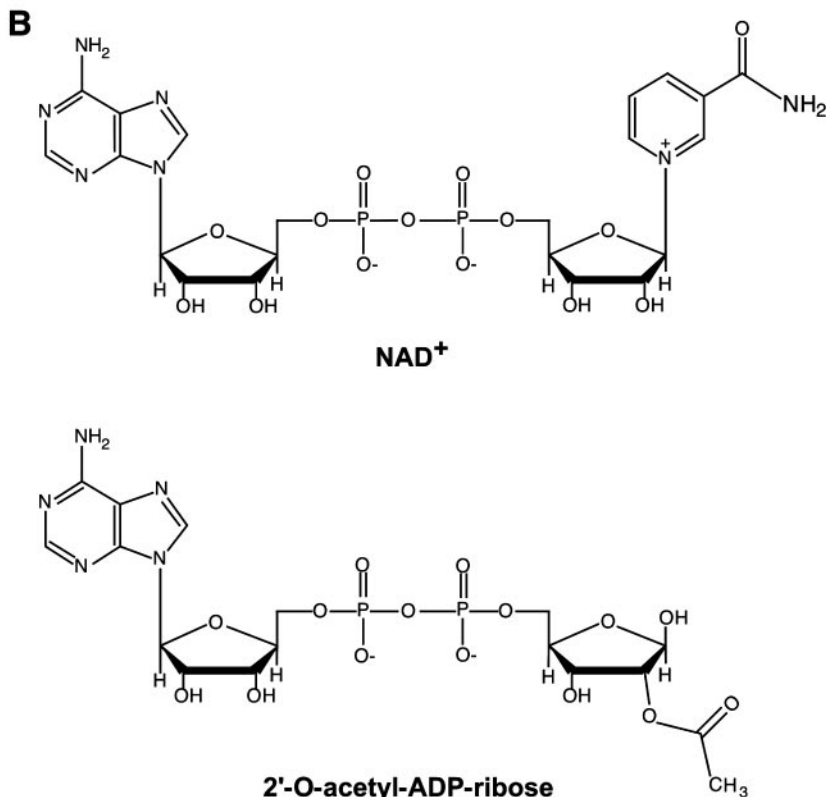
**A**

NAD + acetyl-K-histone $\xrightarrow{\text{Sir2}}$ NAM + O-acetyl-ADP-ribose + K-histone

**B**



NAD$^+$

2'-O-acetyl-ADP-ribose

**Figure 1** Coupling of deacetylation to NAD cleavage and acetyl transfer from substrate to ADP-ribose by Sir2 and Sir2-like proteins. (*A*) The overall reaction scheme. (*B*) Structures of NAD$^+$ and 2'-O-acetyl-ADP-ribose, the novel compound produced by Sir2 and Sir2-like proteins, are shown. NAM = nicotinamide.

An important clue for the molecular mechanism of Sir2 activity came from the stoichiometry between deacetylation and NAD$^+$ breakdown. For each acetyl lysine that was deacetylated by Sir2, one NAD$^+$ molecule was cleaved (17, 19, 20) to produce unexpected products, nicotinamide and O-acetyl-ADP-ribose, instead of the predicted nicotinamide and ADP-ribose (Figure 1) (19, 20). The O-acetyl-ADP-ribose was generated from the transfer of an acetyl group from substrate to the ADP-ribose moiety of NAD$^+$ (19, 20). It was clear that Sir2 has two coupled enzymatic activities, deacetylation and NAD$^+$ breakdown, that

produce a new compound, O-acetyl-ADP-ribose. These two enzymatic activities and the novel enzymatic reaction product raise important questions: Why is an energetically favorable reaction (deacetylation) coupled to $NAD^+$ hydrolysis, and what is the biological role of O-acetyl-ADP-ribose?

The $NAD^+$ dependence of deacetylation by Sir2 may possibly provide regulation of the biological functions of the Sir2, such as gene silencing, metabolism, and aging. It was proposed that O-acetyl-ADP-ribose has a unique cellular function that may be linked to the Sir2 gene-silencing effect, raising the possibility that this product had an important signaling role as a cofactor for its catalytic activity (20). In support of this notion, a quantitative microinjection of exogenous O-acetyl-ADP-ribose into starfish oocytes delayed oocyte maturation (21). Interestingly, a group of enzymes, the Nudix hydrolase family, can hydrolyze O-acetyl-ADP-ribose (22).

## STRUCTURE OF THE SIR2 FAMILY PROTEINS

The structures of Sir2 homologs have been determined (archaeal Sir2 homolog sir2-Af1, human SIRT2, and archaeal sir2-Af2). Here, we discuss the Sir2 structure and the implications of the structure in our understanding of the Sir2 family.

The structure of an archaeal Sir2 homolog sir2-Af1 revealed that the protein consists of a large domain of a classical open $\alpha/\beta$, Rossmann-fold structure and a small domain of $\alpha$ three-stranded antiparallel $\beta$-sheet, two $\alpha$-helices, and a long loop (23). The structure of the human SIRT2 was later solved and compared to that of the sir2-Af1. The two enzymes shared a similar domain architecture. The large domains had an identical topology but only part of the Rossmann folds could be aligned (24). One $NAD^+$ molecule was bound in a pocket between two domains, and it adopted an extended conformation, which was found in Rossmann-fold $NAD^+$-bound proteins. Moreover, the $NAD^+$ molecule was oriented in an inverted orientation in contrast to other $NAD^+$-bound proteins. In the open conformation, the large domain formed the floor, and the small domain formed the ceiling of the $NAD^+$-binding pocket. The majority of the SIR2 family members contain a motif of Cys-X-X-Cys-(X)15–20-Cys-X-X-Cys in the conserved domain, which binds to $Zn^+$ ion (23).

The structure of archaeal sir2-Af2 complexed with an acetylated p53 peptide was solved. The p53 peptide lies in the large groove between the Rossmann fold and the small domain. The N terminus of the peptide was close to the zinc-binding domain, and the C terminus was close to the flexible loop region (Figure 2) (25). The key Sir2-Af2 peptide-binding residues were conserved in other Sir2 proteins. The side chain of the acetyl-lysine residue (K-Ac382) fits into a tunnel that led to the $NAD^+$ binding site. Two amino acids at the N terminus from the Ac-Lysine and five amino acids C-terminal
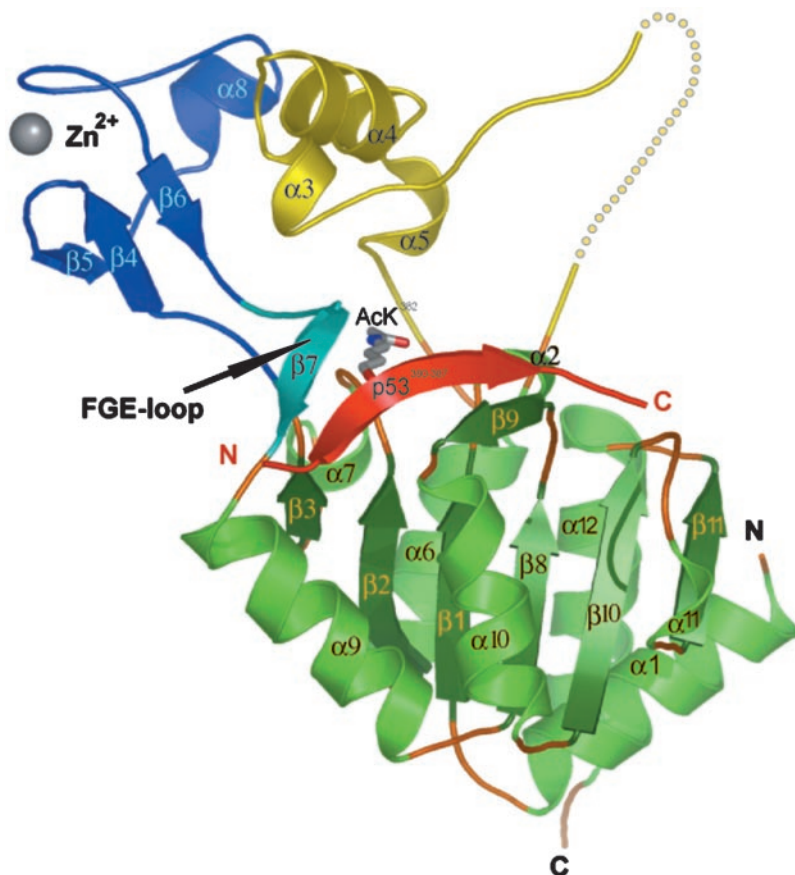
**Figure 2** Cartoon representation of the overall structure of the Af2 and p53 acetylated peptide complex. The reverse Rossmann fold is shown in green and orange. The helical module is shown in yellow, and the disordered region represented as small circles. The zinc-binding module is shown in dark blue, the FGE loop in cyan, and the p53-KAc382 peptide in red.

to the Ac-Lysine of p53 peptide were included in the electron density map. The dominant role of peptide backbone hydrogen bound in substrate binding and the limited extent of substrate side chain burial result in only weak selectivity for certain side chains (25).

In conclusion, the Sir2 solved structure adds to our understanding of the Sir2 family. One issue that has not been explained by the structure is the substrate specificity of the Sir2 enzyme. To understand this issue, other techniques, such as identification of the in vivo substrate by two-dimensional acetylated gels or peptide library screening, may be utilized.

## INHIBITORS AND ACTIVATORS

Several different chemical compounds have been shown to inhibit the deacetylase activity of the Sir2 family. One inhibitor, splitomicin, was identified in a 6000 chemical compound screen. Treatment with splitomicin disrupted silencing at the HML, HMR, and telomeric loci of the budding yeast. Interestingly, transcriptional profiles of splitomicin-treated cells mimic those of a *sir2* mutant. Splitomicin inhibits the $NAD^+$-dependent deacetylase activity of Sir2 in vitro (26).

Another inhibitor was identified in a high-throughput phenotypic screen. This inhibitor, sirtinol is derived from 2-hydroxy-1-napthaldehyde. Sirtinol interferes with body axis formation in *Arabidopsis* (27).

Nicotinamide, a product of the Sir2 deacetylation reaction, is an inhibitor of Sir2 activity both in vivo and in vitro. In yeast cells, exogenous nicotinamide derepresses all three Sir2 target loci, increases recombination at the *rDNA* loci, and shortens life span, comparable to that of the *sir2* mutant (28). Nicotinamide has been shown to inhibit a Sir2 homolog, SIRT1, a negative p53 regulator, promoting p53-dependent apoptosis in mammalian cells (29, 30). To prove that Sir2 was regulated by the changes in nicotinamide levels, Anderson et al. (31) showed that the *PNC1,* which encodes an enzyme that deaminates nicotinamide, was both necessary and sufficient for life span extension by depleting nicotinamide (Figure 3, yeast). Characterization of the base-exchange reaction revealed that nicotinamide regulates Sir2 activity by switching between deacetylation and base exchange (32).

Small molecules that stimulate the SIRT1 deacetylase activity were recently identified. Two of these activators, quercetin and piceatannol, are structurally similar to each other. These compounds stimulate SIRT1 activity by more than severalfold. A secondary screen within the quercetin and piceatannol family identified 15 additional SIRT1 activators. The most potent activator was resveratrol, a polyphenol found in red wine, which is implicated in a number of health benefits. In vitro, resveratrol lowers the $K_m$ values for the acetylated peptide and $NAD^+$ by 35 and 5 times, respectively. At a low concentration, resveratrol increased the yeast life span by 70%, whereas a high concentration had only partial effect. Resveratrol did not further extend the life span of caloric restricted yeast, indicating that they probably act through the same pathway. In human cells, treatment with a low concentration of resveratrol increased cell survival following DNA damage. Moreover, a low resveratrol concentration decreased the acetylation of p53 at the lysine residue 382, a known SIRT1 substrate; however, a high concentration caused the opposite (33).

In conclusion, a large number of chemical activators and inhibitors were identified in the recent years. Those compounds may help us to further understand Sir2 biology.
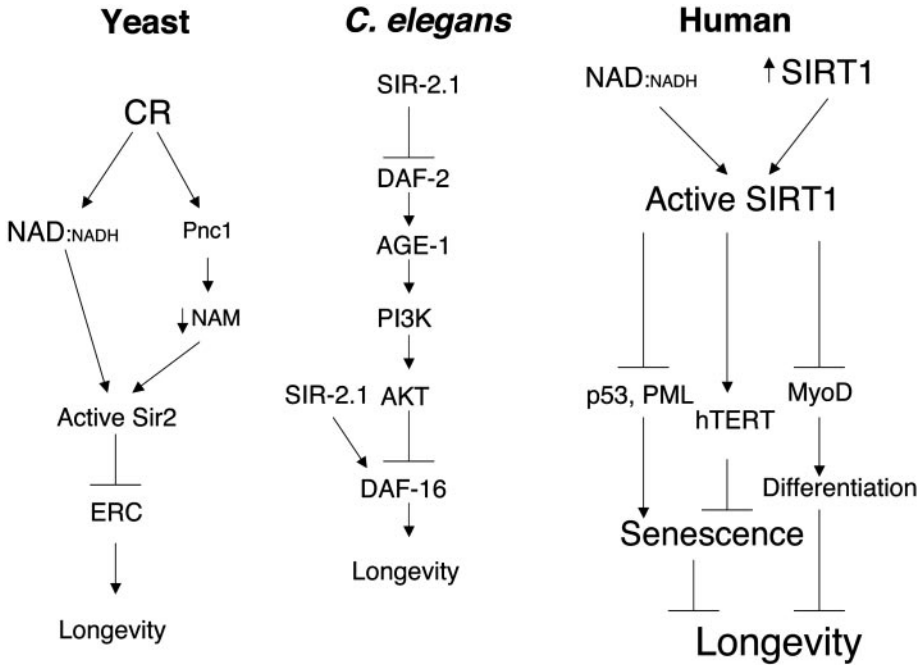
**Figure 3**  Model showing how Sir2 may extend the life span of yeast, *Caenorhabditis elegans*, and humans. The role of Sir2 in these organisms is discussed in the text.

## CELLULAR LOCALIZATION, PROTEIN INTERACTIONS AND PROTEIN SUBSTRATES

The amount of data on the cellular localization, protein interactions, and substrates of the Sir2 family members are rapidly increasing. In Table 1, we summarize the current knowledge of the most important Sir2 family members. In Figure 4, we aligned the sequences of Sir2 substrates, in order to find a consensus sequence.

### Yeast Sir2

SIR2 AS A DEACETYLASE     Since Sir2 was discovered to be an $NAD^+$-dependent deacetylase, significant progress in the Sir2 substrates and Sir2 chromatin binding sites has been made. In this section, we summarize the recent discoveries. To learn more about the yeast Sir2, you may read other recent reviews (34–37).

The Sir2 enzymatic activity is not required for the initial binding, but it is important for the association with the regions distal to the nucleation sites. At the *rDNA* sites, histone H4 was hypoacetylated in a Sir2-dependent manner (38).

With a genome-wide acetylation microarray, Gruastein and colleagues (39) showed that a *SIR2* deletion leads to hyperacetylation of subtelomeric regions,

**TABLE 1**  Sir2 family members

| Gene | Subcellular localization | Interactors | Targets | Activity |
|---|---|---|---|---|
| Sir2 (yeast) | Nucleolus (86) | RAP1 (87) | Histone H3 (K9) (K14) | Deacetylase (16) |
| | Telomeres (77) | SIR4 (88) | H4 (K16) (16) | |
| | | Net1 (89) | | |
| *CobB* | | | Acetyl-CoA synthetase (44) | Deacetylase (44) |
| Archaeal Sir2 | | Alba (46) | Alba (46) | Deacetylase ADP ribosyl transferase (46) |
| dSIR2 (*Drosophila*) | Nucleus (47) | Hairy (48) | Histone H4 (K5) (K8) (K12) (K16) | Deacetylase (47, 48) |
| SIRT1 | Nucleus (29, 30) PML bodies (57) | p53 (29, 30) PML (57) PCAF/GCN5 (53) CTIP2 (63) HES1 (62) | Histone H3 (K9 & 14) H4 (K16) (16) p53 (k382) (29, 30) TAFI68 (61) PCAF/MyoD (53) | Deacetylase (16) |
| SIRT2 | Cytoplasm (68, 69) | HDAC6 (68) | Tubulin (68) | Deacetylase (68, 69) |
| SIRT3 | Mitochondria (72, 90) | | | Deacetylase (72, 90) |
| SIRT4 | | | | Not active (68) |
| SIRT5 | | | | Deacetylase (68) |
| SIRT6 | | | | Not active (68) |
| SIRT7 | | | | Not active (68) |

less than 4 kb from the telomeric ends, the mating-type loci (*HML* and *HMR*), and the *rDNA* loci.

Deletion of *SIR2* caused an increase in histone H3 and H4 acetylation within the rDNA region. The largest increase in acetylation occurred on histone H3 (40).

Two Sir2-containing protein complexes were identified via biochemical purification. One complex includes Sir4 and is active as an $NAD^+$-dependent histone deacetylase, whereas another complex contains Net1, which is a part of nucleolar silencing and the telophase exit (RENT) complex and recruits Sir2 to the *rDNA* loci, and possesses a deacetylase activity that is only partially dependent on $NAD^+$. Both complexes efficiently bind to nucleosomes (41). All of these data

## Sir2 deacetylation consensus sequences

| | |
|---|---|
| H3(9) | ARTKQTAR**K**STGG**K**APRKQLATKAA |
| H3(14) | ARTKQTAR**K**STGG**K**APRKQLATKAA |
| H4(16) | AGG**K**GG**K**GMGAK**V**GA**K**RHS |
| P53(382) | HL**K**S**K**KGQSTS**R**HK**K**LMF**K**TEGPDSD |
| TUBULIN | MER**LS**VDYG**K**KSKLEFSIYP |
| Alba | MSSGTPTPSNVVLIG**K**K |
| Consensus | K(V/S)(V/T)GG**K**KXX**K** |

**Figure 4**  Sir2 family deacetylation target consensus sequences. The amino acid sequence of the Sir2 targets are shown: H3(9), histone H3 lysine 9; H3(14), histone H3 lysine 14; H4(16), histone H4 lysine 16; p53(382), p53 lysine 382; TUBULIN, tubulin; and Alba, alba. The Sir2 target consensus sequence is indicated.

indicate that, in budding yeast, the histone deacetylation is the major biological function of Sir2.

*SCHIZOSACCHAROMYCES POMBE* Sir2    The *Schizosaccharomyces pombe* Sir2 is the closest Sir2 homolog. Similar to *Saccharomyces cerevisiae* Sir2, it is an NAD$^+$-dependent deacetylase with histone H3 lysine 9 and H4 lysine 16 as substrates. In vivo the *S. pombe* Sir2 regulates silencing at the donor mating-type loci, telomeres, and the inner centromeric repeats but not at the *rDNA* loci. These results suggest that the molecular function of the Sir2-dependent silencing involves the deacetylation of histone H3 lysine 9 in chromatin (42).

## Bacterial Sir2

It is remarkable that SIR2 genes are conserved not only in all eukaryotic organisms examined, but also in prokaryotes and archaea. Below we describe features of the noneukaryotic SIR2 proteins.

*SALMONELLA* Sir2 (*CobB*)    *CobB* complements the lack of phosphoribosyltransferase activity in a *cobT* mutant. *CobB* catalyzes the synthesis of N-(5-phospho-α-D-ribosyl)5,6-dimethylbenzimidazole, a cobalamin biosynthetic intermediate, from nicotinate mononucleotide and 5,6-dimethylbenzimidazole (13). Recently, *CobB* was shown to be required for the acetate and propionate activation via the high-affinity acyl-CoA synthetase pathway. The acyl-CoA synthetase is acetylated on lysine 609 (43), which is an essential residue for catalysis (44). *CobB* deacetylates the acyl-CoA synthetase in an NAD$^+$-dependent manner to activate the enzyme.

ARCHAEAL Sir2    The archaeal *Sulfolobus solfataricus* P2 encodes a single Sir2 homolog, ssSIR2 (45). The ssSIR2 is an active $NAD^+$-dependent deacetylase as well as a mono-ADP-ribosyl transferase (46). The archaeal protein Alba, which is a major *Sulfolobus* chromatin protein, interacts with the ssSir2. Alba was doubly acetylated at the $\alpha$-amino group of the N terminus and at either lysine 16 or 17 of the protein. Lysine to alanine mutations at these residues resulted in a significant decrease in the DNA binding affinity of Alba and less repression of the transcription in vitro. The acetylated Alba also has a lower DNA affinity in vivo. ssSIR2 deacetylates Alba, which increases DNA affinity and thereby represses transcription (46).

## Drosophila Sir2

Five *Drosophila* genes belong to the Sir2 family. Of these, dSir2 is the closest homolog of the yeast Sir2. dSir2 is an active deacetylase in vitro, capable of deacetylating histone H4 (47–49). In vivo dSir2 was shown to be a requirement for hetrochromatic silencing. Moreover, dSir2 genetically and physically interacted with Hairy, a bHLH euchromatic repressor and key regulator of *Drosophila* development (48). The dSir2 mRNA was detected during the first 2 h of embryogenesis, indicating that the transcripts are maternally derived (49). In the adult fly, the protein is primarily nuclear (49). In the embryo, prior to nuclear cycle 12, it is detected both in the nucleus and in the cytoplasm. By the syncytial blastoderm stage (cycle 13), dSir2 was only in the cytoplasm. As cellularization begins (cycle 14), it appeared again both in the nucleus and the cytoplasm (48). Contrary to what had been reported previously (48), Astrom et al. (50) found that a dSir2 knockout fly has no effect on viability, developmental rate, and sex ratio. In agreement with the previous report, they observed a modest effect on position effect variegation (50). Notably, they showed that dSir2 knockout results in the shortening of the fly life span (50).

## Mammalian Sir2

The mammalian Sir2 gene family is comprised of seven members. Each is defined by a conserved core domain, and some contain additional N- or C-terminal sequences. Whereas much is known about SIRT1, less is known about the other six mammalian sir2 homologues.

SIRT1    The human Sir2 ortholog SIRT1 is an $NAD^+$-dependent deacetylase (16). The SIRT1 protein is localized in the nucleus (29, 30). SIRT1 interacts with and deacetylates a growing number of proteins. A knockout mouse showed that this protein is important for the embryonic development (51, 52), and recently it was shown that it plays a role in muscle differentiation (53). Moreover, when overexpressed, SIRT1 appears to increase the *hTERT* expression (54).

p53 INTERACTION     Following DNA damage, p53 is acetylated and activated by p300 acetyltransferase. (55). Moreover, the HDAC1 is capable of deacetylating and repressing p53 (56). SIRT1 was hypothesized as playing a role in the p53 pathway as well. To test this possibility the physical interaction between SIRT1 and p53 was investigated. Vaziri et al. (29) and Luo et al. (56) showed that p53 and SIRT1 coimmunoprecipitate. DNA-damaging agents augment in vivo interaction (30). In vitro a p53 peptide, acetylated at lysine 382, served as a substrate for SIRT1. Importantly, $NAD^+$ was required for the deacetylation reaction (30, 57). Upon exposure of immortalized human fibroblast to ionizing radiation, a marked increase in the p53 acetylation level was detected. The increase in the acetylation levels was abrogated in the cells that overexpress the *SIRT1* protein (29). In vivo deacetylation of p53 was inhibited by nicotinamide (30). The biological consequences of the deacetylation are the repression of the p53-dependent transcription and apoptosis (29, 30, 57). Also, a SIRT1 point mutation in the conserved deacetylase motif inhibited p53 deactylation as a dominant negative and activated the p53-dependent apoptosis (29). Recently, a SIRT1 knockout mouse was shown to have highly acetylated p53, and it induced apoptosis in thymocytes (52).

PML INTERACTION     The nuclear bodies (NB), often termed promyelocytic leukemia protein (PML) NB, are distinct nuclear substructures that accumulate PML proteins (58). A typical cell contains 10–30 PML NB per nucleus. The PML interacts with CBP and HDAC1, which led the Kouzarides group (57) to explore possible interactions between SIRT1 and PML4. Indeed, endogenous SIRT1 interacts with PML4. When SIRT1 was overexpressed with PML4, it localizes to the PML NB (57). Moreover, SIRT1 and PML4 colocalizes with p53 in the PML NB. Overexpression of PML4 in primary cells leads to an immediate growth arrest. Interestingly, SIRT1 overexpression rescued the cells from the growth arrest (57). Together, these results indicate that SIRT1 may be a positive effector of cell growth that negatively regulates p53 and PML.

BCL6 INTERACTION     BCL6 is a nuclear protein, which belongs to the BTB/POZ family of zinc finger transcription factors. It primarily functions as a transcriptional repressor (59). BCL6 plays a role in the control of lymphocyte activation, differentiation, and apoptosis. p300 binds to and acetylates BCL6, inhibiting its activity as a transcriptional repressor. Inhibition of classes I and II HDACs by trichostatin A and inhibition of SIR2 family (class III HDAC) by nicotinamide leads to an increase in the BCL6 acetylation levels (60). These results suggest that one of the SIR2 family members deacetylates BCL6, represses the transcription of BCL6 target genes, and consequently inhibits B cell differentiation.

TAF$_I$68 INTERACTION     TAF$_I$68 is the second largest subunit of the RNA polymerase I complex that binds to the TATA box and is acetylated by PCAF. In vitro TAF$_I$68 acetylation stimulates RNA polymerase transcription (61). The mouse

SIRT1 deacetylates TAF$_I$68 in vitro, and it may thereby repress the polymerase I transcription apparatus (61).

HES1 INTERACTION    The Hairy related bHLH protein functions as a transcriptional repressor and plays an important role in diverse aspects of development. HES1 and the HEY2 are human Hairy homologs. SIRT1 associates with both HES1 and HEY2 in vivo. In vitro SIRT1 interacts with the bHLH domain of HES1. However, SIRT1 augments only slightly the repression mediated by HES1 and HEY2 (62). The biological implication of this interaction needs to be studied.

CTIP2 INTERACTION    CTIP2 is a sequence-specific DNA binding protein that represses transcription via direct DNA binding. In vivo and in vitro SIRT1 binds to CTIP2 and is recruited to CTIP2 target promoters in a CTIP2-dependent manner. SIRT1 stimulates the repression by CTIP2 and enhances the histone deacetylation of CTIP2 target promoters (63). These data suggest that SIRT1 can be recruited to promoters by specific transcription factors and function to repress the transcription of specific genes.

KNOCKOUT MICE    Two different groups created two independent lines of the *SIRT1* knockout mice. Both groups showed that the SIRT1 knockout mice are viable. However, the birth ratio of the homozygous knockout mice to other animals was lower than expected. The lower survival of the null animals at birth reflects the immediate postnatal loss of abnormal fetuses. The mice were smaller than the wild-type littermates, and most of them died during the first few months after birth (51, 52). One of the most obvious developmental defects was the delay in eyelid opening. In the *SIRT1* null mice, eyelids stayed closed for at least several months after birth. Eyes of the *SIRT1* knockout mice were smaller and irregularly shaped (52). This observation indicates that the *SIRT1* may have an important role in eye development. McBurney et al. (51) found that the lung and pancreas were affected in the mutant mice. The pancreas showed patchy atrophy of the exocrine epithelia (51). The *SIRT1* knockout mice made by the Alt group (52) showed a cardiac defect but did not show lung and pancreas defects. The *SIRT1* transcript is widely expressed in many tissues and particularly evident in testis and ovary. Interestingly, both sexes of the null animals are sterile. The female had smaller ovaries in which corpora lutea were conspicuously absent and the wall of uterus was thin. The authors (51) showed that the sterility was due to a hormonal inadequacy. The male null mice had a dramatically reduced number of mature sperms. Importantly, none of the *SIRT1* null sperms were motile nor had a normal morphology (51). The level of p53 acetylation was much higher in the knockout mice. In contrast to the previous reports, the hyperacetylation was not specific to lysine 379 but also occurred at lysine residues 317 and 370. Following DNA damage, the steady-state level of p53 was not induced, and p21 was not induced in knockout mouse embryonic fibroblasts (MEFs). However, apoptosis in thymocytes was elevated in the mutant mice (52).

REGULATION OF SKELETAL MUSCLE DIFFERENTIATION    The expression of muscle-cell-specific genes is regulated by acetylation and deacetylation of transcription factors (64). The muscle transcriptional regulator MyoD is an acetylated protein. In a recent study, the Sartorelli group (64) showed that the mouse SIRT1 negatively regulates skeletal muscle differentiation. By using a battery of Sir2 family inhibitors, they demonstrated that these inhibitors activated the transcription of muscle-specific reporters. Furthermore, SIRT1 overexpression negatively regulated the transcription of those genes and inhibits differentiation into muscle cells. SIRT1 directly interacted with the PCAF/GCN5 acetyltransferases in vitro and in muscle cells. PCAF mediated the interaction between SIRT1 and MyoD. In vitro SIRT1 deacetylates MyoD and PCAF in an $NAD^+$-dependent manner. With microarray experiments, the authors (64) showed that myogenin and MEF2C expression were negatively regulated by SIRT1. They further showed that many genes that were activated by MyoD and involved in myogenesis were repressed by SIRT1. In addition, by chromatin immunoprecipitation, they found that the SIRT1 was recruited to the MyoD targets and deacetylased histones in the target promoters. Notably, they explored whether the $NAD^+$/NADH ratio, which decreases during muscle differentiation, regulates SIRT1 deacetylase activity. Indeed, they found that the change in the ratio regulates SIRT1 enzymatic activity (53).

SIRT2    SIRT2 is another member of the Sir2 family. In the phylogenic tree, it is localized to the same branch as Sir2 and SIRT1 (65). A Northern blot analysis showed that *SIRT2* is highly expressed in heart, brain, testis, and skeletal muscle (66, 67). The protein is cytoplasmic (66–68), and its levels are regulated during the cell cycle. The SIRT2 protein level increases dramatically during mitosis, and it becomes phosphorylated at the G2/M transition (69). SIRT2 overexpression dramatically prolongs the M phase. Moreover, SIRT2 is targeted for degradation by the proteasome (69).

Reversible acetylation of tubulin had been implicated in the regulation of microtubule stability and function (70). Interestingly, the HDAC6 was shown to deacetylate tubulin (71). The Verdin group (68) showed that SIRT2 protein colocalizes with microtubules. They showed that SIRT2 deacetylates lysine 40 of $\alpha$-tubulin in vivo and in vitro and that SIRT2 *RNAi* results in hyperacetylation of tubulin. They further demonstrated that SIRT2 and HDAC6 coimmunoprecipitate in vivo (68). In contrast to HDAC6, which regulates microtubule-dependent cell motility, the biological implication of tubulin deacetylation by SIRT2 is not known.

SIRT3    SIRT3 is another member of the Sir2 family. In the phylogeny tree, SIRT3 is localized to the same branch as Sir2 and SIRT1, and it is the closest paralog of SIRT2 (65). A Northern blot analysis demonstrated that the gene is highly expressed in brain, heart, liver, kidney, testis, and muscle (66, 72). The SIRT3 protein is localized to the mitochondrial matrix (90). The N terminus of the protein is proteolytically processed in the mitochondrial matrix to form a mature product. The unprocessed SIRT3 is enzymatically inactive, but following

signal peptide cleavage, it becomes active as a histone deacetylase (72, 90). Biological targets of this protein are not yet known.

SIRT4    SIRT4 is another member of the Sir2 family. Other than SIRT5, SIRT4 is the most distant from Sir2 and SIRT1 in phylogeny tree (65). RT-PCR analysis of SIRT4 expression from adult and fetal tissues showed that the gene is broadly expressed in all tissues, except leukocytes in the adult and thymus in the fetus (14). SIRT4 may be enzymatically inactive as a histone deacetylase in vitro (68), and a biological role is yet to be discovered.

SIRT5    SIRT5 is is the closest homolog of the bacterial *CobB* and is the most distant from Sir2 and SIRT1 (65). RT-PCR analysis of SIRT5 expression profiles from adult and fetal tissues demonstrated that the gene is broadly expressed (14). SIRT5 is enzymatically active as a histone deacetylase (68), and its biological role is yet to be discovered.

SIRT6    SIRT6 is another member of the Sir2 family, and it is the closest SIRT7 homolog (65). SIRT6 is enzymatically inactive as a histone deacetylase (68), and its biological role is yet to be discovered.

SIRT7    SIRT7 is highly expressed in the spleen, ovary, and thyroid. SIRT7 is also highly expressed in thyroid carcinomas when compared to normal thyroid tissues. In contrast, its expression is almost undetectable in adenomas and normal thyroid tissues (73, 74). SIRT7 is enzymatically inactive as a histone deacetylase (68).

## SIR2 AND AGING

The yeast *Saccharomyces cerevisiae* divides asymmetrically to give rise to a larger mother cell and a smaller daughter cell. In this organism, life span can be defined by the number of cell divisions undergone by a mother cell before it stops dividing (75). In a screen to isolate long-living mutants, Kennedy et al. (76) found eight strains that exhibited extended life span. One of them, *SIR4−42*, was a *SIR4* mutant that lacks a C-terminal domain (76). The life span extension by the Sir4−42 allele was dominant, and mutations in the SIR2 or the SIR3 genes, which are part of the SIR complex, abolished the life span extension by the SIR4−42. In a further characterization, the *SIR4−42* gene product changed the localization of the SIR complex from the telomeres to the nucleolus (77). Interestingly, in old yeast mother cells, the Sir3 translocated from the telomeres to the nucleolus (77), suggesting that something in the nucleolus might regulate the yeast life span. Deletion of either the *SIR3* or *SIR4* resulted in a 20% decrease in mean life span, and it was due to simultaneous expression of the two mating-type genes. A *sir2* mutant resulted in much shorter life span (approxi-

mately 50% of the control) (78). The life span shortening by *sir2* mutants was due to the increase in homologous recombination at the *rDNA* loci, which results in the formation of an extrachromosomal *rDNA* circle (ERC) in the nucleolus (Figure 3, yeast). Interestingly, introduction of a second copy of the *SIR2* into the yeast genome extended the replicative life span by 30% (78). Therefore, the Sir2 protein is a limiting factor of yeast life span. Mutations that abolish the Sir2 deacetylase activity shorten life span (16). Interestingly, SIR2 is the only yeast Sir complex member that is conserved through evolution.

Tissenbaum & Guarente (79) decided to look at the effect of overexpression of a Sir2 homolog on *Caenorhabditis elegans* life span. The cross of a sir-2.1 overexpression strain with a *daf-16* mutant strain, a downstream target of the insulin pathway that shortens life span when mutated, abolished the life span extension by the sir-2.1. Also, a cross with a daf-2 mutant, an insulin receptor, resulted in no further extension of life span (79) (Figure 3, *C. elegans*). Thus, sir-2.1 overexpression significantly extended the life span for *C. elegans* (79). The life span extension by the *sir-2.1* overexpression is via the insulin pathway, which was already established in regulating its life span [for review, see (80)]. It is remarkable that both replicative aging in yeast and postmitotic aging in the worm are regulated by Sir2. It will be interesting to determine whether Sir2 regulates the aging process in higher eukaryotes as well.

## CALORIC RESTRICTION

Caloric restriction (CR) refers to a dietary regime, low in calories without undernutrition. CR extends the life span in many organisms, which include rotifers, spiders, worms, fish, mice, and rats (81). Recent data suggest that it may be true for primates as well (82). Although it has been suggested that CR might work by reducing the levels of reactive oxygen species during respiration, the mechanism of life span extension was uncertain. Lin et al. (83) showed that yeast cells exhibited a longer life span on 0.5% glucose-containing media than on 2% glucose-containing media, suggesting that CR in yeast extends life span. Limiting the availability of glucose by mutating a glucose transporter or blocking its downstream signaling pathway also extends the life span. Thus, reduction in glucose concentration extends life span and provides a model for CR in yeast (83). By using a mutant strain that mimics reduction in glucose concentration, Lin et al. demonstrated that the yeast life span extension by CR requires *SIR2* (83, 84) (Figure 3, yeast). As mentioned before, Sir2 is an $NAD^+$-dependent enzyme. $NAD^+$ may be a sensor that activates Sir2 during CR. In fact, *NPT1,* involved in de novo synthesis of $NAD^+$, was required for CR-dependent life span extension. The life span extension by CR in yeast is likely caused by the reduction in rDNA circles by Sir2 (83).

Next, the mechanism by which CR increases Sir2 activity and extends life span was explored. A clue came from the glucose metabolism. Glucose is

metabolized to pyruvate, where the pathway flows into either respiration or fermentation, depending on $O_2$ availability. Respiration generates 36 ATP molecules per glucose, whereas fermentation generates only two ATP molecules. Indeed, respiration was activated during CR (84). The shift toward respiration was necessary for life span extension (84). Following this shift, the $NAD^+$/NADH ratio widely changes to favor the activation of Sir2, resulting in the inhibition of recombination at the rDNA locus and the extension of life span (Figure 3, yeast).

## CONCLUSION

How do these findings relate to human aging? In mammals, the Sir2 enzymatic activity may be regulated by changes in the steady-state protein levels, the $NAD^+$:NADH ratio, or the nicotinamide (NAM) levels (Figure 3, human). The Sartorelli group (53) showed that the $NAD^+$/NADH ratio decreases during muscle differentiation and thus regulates SIRT1 deacetylase activity (Figure 3, human). Activation of SIRT1 could then inhibit cell senescence by repressing p53 (29, 30, 57), repressing PML (57), and activating hTERT (54) (Figure 3, human). In addition, active SIRT1 would stall differentiation of muscle by repressing MyoD (53) (Figure 3, human) and perhaps exert similar effects on other tissues. It will be fascinating to observe additional functions of the mammalian SIR2 homologs when they are discovered.

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## LITERATURE CITED

1. Rine J, Herskowitz I. 1987. *Genetics* 116: 9–22
2. Gottschling DE, Aparicio OM, Billington BL, Zakian VA. 1990. *Cell* 63:751–62
3. Bryk M, Banerjee M, Murphy M, Knudsen KE, Garfinkel DJ, Curcio MJ. 1997. *Genes Dev.* 11:255–69
4. Smith JS, Boeke JD. 1997. *Genes Dev.* 11:241–54
5. Loo S, Rine J. 1994. *Science* 264: 1768–71
6. Bi X, Broach JR. 1997. *Mol. Cell. Biol.* 17: 7077–87
7. Thompson JS, Ling X, Grunstein M. 1994. *Nature* 369:245–47
8. Hecht A, Laroche T, Strahl-Bolsinger S, Gasser SM, Grunstein M. 1995. *Cell* 80: 583–92

9. Braunstein M, Sobel RE, Allis CD, Turner BM, Broach JR. 1996. *Mol. Cell. Biol.* 16:4349–56

10. Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR. 1993. *Genes Dev.* 7:592–604

11. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. 1997. *Nature* 389:251–60

12. Brachmann CB, Sherman JM, Devine SE, Cameron EE, Pillus L, Boeke JD. 1995. *Genes Dev.* 9:2888–902

13. Tsang AW, Escalante-Semerena JC. 1998. *J. Biol. Chem.* 273:31788–94

14. Frye RA. 1999. *Biochem. Biophys. Res. Commun.* 260:273–79

15. Tanny JC, Dowd GJ, Huang J, Hilz H, Moazed D. 1999. *Cell* 99:735–45

16. Imai S, Armstrong CM, Kaeberlein M, Guarente L. 2000. *Nature* 403:795–800

17. Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, et al. 2000. *Proc. Natl. Acad. Sci. USA* 97:5807–11

18. Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, et al. 2000. *Proc. Natl. Acad. Sci. USA* 97:6658–63

19. Tanny JC, Moazed D. 2001. *Proc. Natl. Acad. Sci. USA* 98:415–20

20. Tanner KG, Landry J, Sternglanz R, Denu JM. 2000. *Proc. Natl. Acad. Sci. USA* 97:14178–82

21. Borra MT, O'Neill FJ, Jackson MD, Marshall B, Verdin E, et al. 2002. *J. Biol. Chem.* 277:12632–41

22. Rafty LA, Schmidt MT, Perraud AL, Scharenberg AM, Denu JM. 2002. *J. Biol. Chem.* 277:47114–22

23. Min JR, Landry J, Sternglanz R, Xu RM. 2001. *Cell* 105:269–79

24. Finnin MS, Donigian JR, Pavletich NP. 2001. *Nat. Struct. Biol.* 8:621–25

25. Avalos JL, Celic I, Muhammad S, Cosgrove MS, Boeke JD, Wolberger C. 2002. *Mol. Cell* 10:523–35

26. Bedalov A, Gatbonton T, Irvine WP, Gottschling DE, Simon JA. 2001. *Proc. Natl. Acad. Sci. USA* 98:15113–18

27. Grozinger CM, Chao ED, Blackwell HE, Moazed D, Schreiber SL. 2001. *J. Biol. Chem.* 276:38837–43

28. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. 2002. *J. Biol. Chem.* 277:45099–107

29. Vaziri H, Dessain SK, Eagon EN, Imai SI, Frye RA, et al. 2001. *Cell* 107:149–59

30. Luo JY, Nikolaev AY, Imai S, Chen DL, Su F, et al. 2001. *Cell* 107:137–48

31. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. 2003. *Nature* 423:181–85

32. Sauve AA, Celic I, Avalos J, Deng H, Boeke JD, Schramm VL. 2001. *Biochemistry* 40:15456–63

33. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, et al. 2003. *Nature* 425:191–96

34. Hekimi S, Guarente L. 2003. *Science* 299:1351–54

35. Sinclair DA. 2002. *Mech. Ageing Dev.* 123:857–67

36. Gasser SM, Cockell MM. 2001. *Gene* 279:1–16

37. Guarente L. 2000. *Genes Dev.* 14:1021–26

38. Hoppe GJ, Tanny JC, Rudner AD, Gerber SA, Danaie S, et al. 2002. *Mol. Cell. Biol.* 22:4167–80

39. Robyr D, Suka Y, Xenarios I, Kurdistani SK, Wang A, et al. 2002. *Cell* 109:437–46

40. Buck SW, Sandmeier JJ, Smith JS. 2002. *Cell* 111:1003–14

41. Ghidelli S, Donze D, Dhillon N, Kamakaka RT. 2001. *EMBO J.* 20:4522–35

42. Shankaranarayana GD, Motamedi MR, Moazed D, Grewal SI. 2003. *Curr. Biol.* 13:1240–46

43. Starai VJ, Takahashi H, Boeke JD, Escalante-Semerena JC. 2003. *Genetics* 163:545–55

44. Starai VJ, Celic I, Cole RN, Boeke JD, Escalante-Semerena JC. 2002. *Science* 298:2390–92

45. She Q, Singh RK, Confalonieri F, Zivanovic Y, Allard G, et al. 2001. *Proc. Natl. Acad. Sci. USA* 98:7835–40

46. Bell SD, Botting CH, Wardleworth BN, Jackson SP, White MF. 2002. *Science* 296:148–51

47. Barlow AL, van Drunen CM, Johnson CA, Tweedie S, Bird A, Turner BM. 2001. *Exp. Cell Res.* 265:90–103

48. Rosenberg MI, Parkhurst SM. 2002. *Cell* 109:447–58

49. Newman BL, Lundblad JR, Chen Y, Smolik SM. 2002. *Genetics* 162:1675–85

50. Astrom SU, Cline TW, Rine J. 2003. *Genetics* 163:931–37

51. McBurney MW, Yang XF, Jardine K, Hixon M, Boekelheide K, et al. 2003. *Mol. Cell. Biol.* 23:38–54

52. Cheng HL, Mostoslavsky R, Saito S, Manis JP, Gu YS, et al. 2003. *Proc. Natl. Acad. Sci. USA* 100:10794–99

53. Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, et al. 2003. *Mol. Cell* 12:51–62

54. Lin SY, Elledge SJ. 2003. *Cell* 113:881–89

55. Abraham J, Kelly J, Thibault P, Benchimol S. 2000. *J. Mol. Biol.* 295:853–64

56. Luo JY, Su F, Chen DL, Shiloh A, Gu W. 2000. *Nature* 408:377–81

57. Langley E, Pearson M, Faretta M, Bauer UM, Frye RA, et al. 2002. *EMBO J.* 21:2383–96

58. Seeler JS, Dejean A. 1999. *Curr. Opin. Genet. Dev.* 9:362–67

59. Chang CC, Ye BH, Chaganti RS, Dalla-Favera R. 1996. *Proc. Natl. Acad. Sci. USA* 93:6947–52

60. Bereshchenko OR, Gu W, Dalla-Favera R. 2002. *Nat. Genet.* 32:606–13

61. Muth V, Nadaud S, Grummt I, Voit R. 2001. *EMBO J.* 20:1353–62

62. Takata T, Ishikawa F. 2003. *Biochem. Biophys. Res. Commun.* 301:250–57

63. Senawong T, Peterson VJ, Avram D, Shepherd DM, Frye RA, et al. 2003. *J. Biol. Chem.* 278:43041–50

64. Sartorelli V, Puri PL, Chang CC, Ye BH, Chaganti RS, Dalla-Favera R. 2001. *Front. Biosci.* 6:D1024–47

65. Frye RA. 2000. *Biochem. Biophys. Res. Commun.* 273:793–98

66. Yang YH, Chen YH, Zhang CY, Nimmakayalu MA, Ward DC, Weissman S. 2000. *Genomics* 69:355–69

67. Afshar G, Murnane JP. 1999. *Gene* 234:161–68

68. North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. 2003. *Mol. Cell* 11:437–44

69. Dryden SC, Nahhas FA, Nowak JE, Goustin AS, Tainsky MA. 2003. *Mol. Cell. Biol.* 23:3173–85

70. Piperno G, LeDizet M, Chang XJ. 1987. *J. Cell Biol.* 104:289–302

71. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, et al. 2002. *Nature* 417:455–58

72. Onyango P, Celic I, McCaffery JM, Boeke JD, Feinberg AP. 2002. *Proc. Natl. Acad. Sci. USA* 99:13653–58

73. de Nigris F, Cerutti J, Morelli C, Califano D, Chiariotti L, et al. 2002. *Br. J. Cancer* 86:917–23

74. Frye R. 2002. *Br. J. Cancer* 87:1479

75. Muller I, Zimmermann M, Becker D, Flomer M. 1980. *Mech. Ageing Dev.* 12:47–52

76. Kennedy BK, Austriaco NR Jr, Zhang J, Guarente L. 1995. *Cell* 80:485–96

77. Kennedy BK, Gotta M, Sinclair DA, Mills K, McNabb DS, et al. 1997. *Cell* 89:381–91

78. Kaeberlein M, McVey M, Guarente L. 1999. *Genes Dev.* 13:2570–80

79. Tissenbaum HA, Guarente L. 2001. *Nature* 410:227–30

80. Tissenbaum HA, Guarente L. 2002. *Dev. Cell* 2:9–19

81. Weindruch R. 1996. *Toxicol. Pathol.* 24:742–45

82. Lane MA, Black A, Handy A, Tilmont EM, Ingram DK, Roth GS. 2001. *Ann. NY Acad. Sci.* 928:287–95

83. Lin SJ, Defossez PA, Guarente L. 2000. *Science* 289:2126–28

84. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, et al. 2002. *Nature* 418: 344–48

85. Deleted in proof

86. Gotta M, Strahl-Bolsinger S, Renauld H, Laroche T, Kennedy BK, et al. 1997. *EMBO J.* 16:3243–55

87. Moretti P, Freeman K, Coodly L, Shore D. 1994. *Genes Dev.* 8:2257–69

88. Strahl-Bolsinger S, Hecht A, Luo K, Grunstein M. 1997. *Genes Dev.* 11: 83–93

89. Straight AF, Shou W, Dowd GJ, Turck CW, Deshaies RJ, et al. 1999. *Cell* 97: 245–56

90. Schwer B, North BJ, Frye RA, Ott M, Verdin E. 2002. *J. Cell Biol.* 158: 647–57