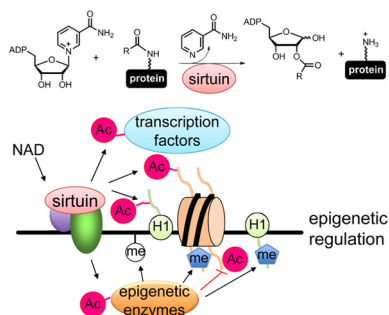# CHEMICAL REVIEWS

Sirtuins in Epigenetic Regulation

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CONTENTS

1. INTRODUCTION

1.1. Discovery of Sirtuins

The founding member of sirtuin is the yeast-silencing information regulator 2 (SIR2) protein, one of four proteins (SIR1−4) required for silencing the mating-type information loci in yeast.[1] SIR2−4, but not SIR1, are also required for gene silencing at telomeres.[2] SIR2 also mediates gene silencing at the rDNA (rDNA) loci, which was shown to be independent of other SIR proteins.[3,4] Immunofluorescence imaging showed that SIR2 is mainly in the nucleolus and telomeres in yeast.[5] It was demonstrated that the silenced genetic loci have low histone acetylation levels compared to loci that are not silenced. Mutation in Sir2−4 increased histone acetylation levels and overexpression of SIR2 but not other SIR proteins led to decreases in histone H4, H2B, and H3 acetylation.[6] Analyzing the sensitivity of yeast chromatin to micrococcal nuclease and dam methyltransferase indicated that Sir2 mutation affects the chromatin structure in the rDNA and mating-type loci.[7]

These interesting discoveries on SIR2 were further elevated by two discoveries made by Guarente and co-workers. The first one was that SIR2 is important for the replicative life span of yeast cells,[8] a finding that was later extended to higher eukaryotic species,[9,10] although the role of sirtuins in life span is highly controversial[11] and may depend on genetic background and diet conditions.[12,13] The second discovery was that SIR2 is an NAD-dependent histone deacetylase (Figure 1),[14] which established SIR2 as a mechanistically novel lysine deacetylase
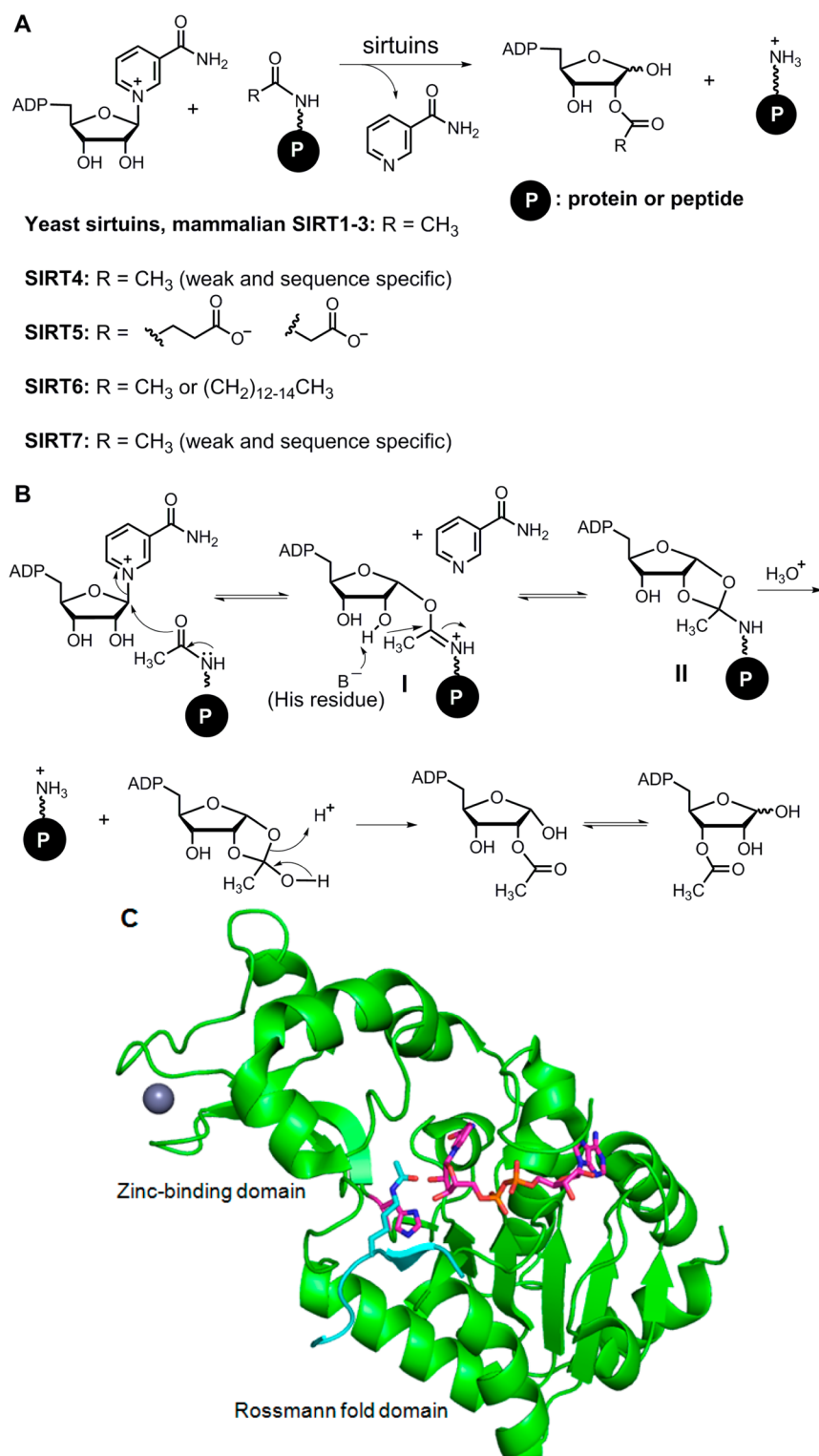
Figure 1. Enzymatic function of sirtuins. (A) NAD-dependent protein lysine deacylation activity of different sirtuins. (B) Enzymatic reaction mechanism of sirtuins. (C) Structure of a ternary sirtuin-NAD-acetyl peptide complex (PDB ID 2H4F). NAD, acetyl lysine, and the key catalytic His residue are shown in stick representation. Bound zinc is shown as a gray sphere. Protein structure picture is generated using PyMol.

and revealed the connection between gene silencing and cellular metabolism. Indeed, it was later found that the life span extension effect of SIR2 is dependent on NAD level and NAD metabolism.[15−17] Several other groups similarly reported the enzymatic activity of SIR2.[18] These landmark discoveries established sirtuins as important players in epigenetics and triggered the explosion of research interest in sirtuins.

1.2. Classification and Enzymatic Activity of Sirtuins

Sirtuins are evolutionarily conserved in all domains of life. On the basis of sequence similarity, sirtuins from different species are classified into at least four classes, classes I−IV.[19] Mammalian SIRT1−3 and all yeast sirtuins belong to class I.
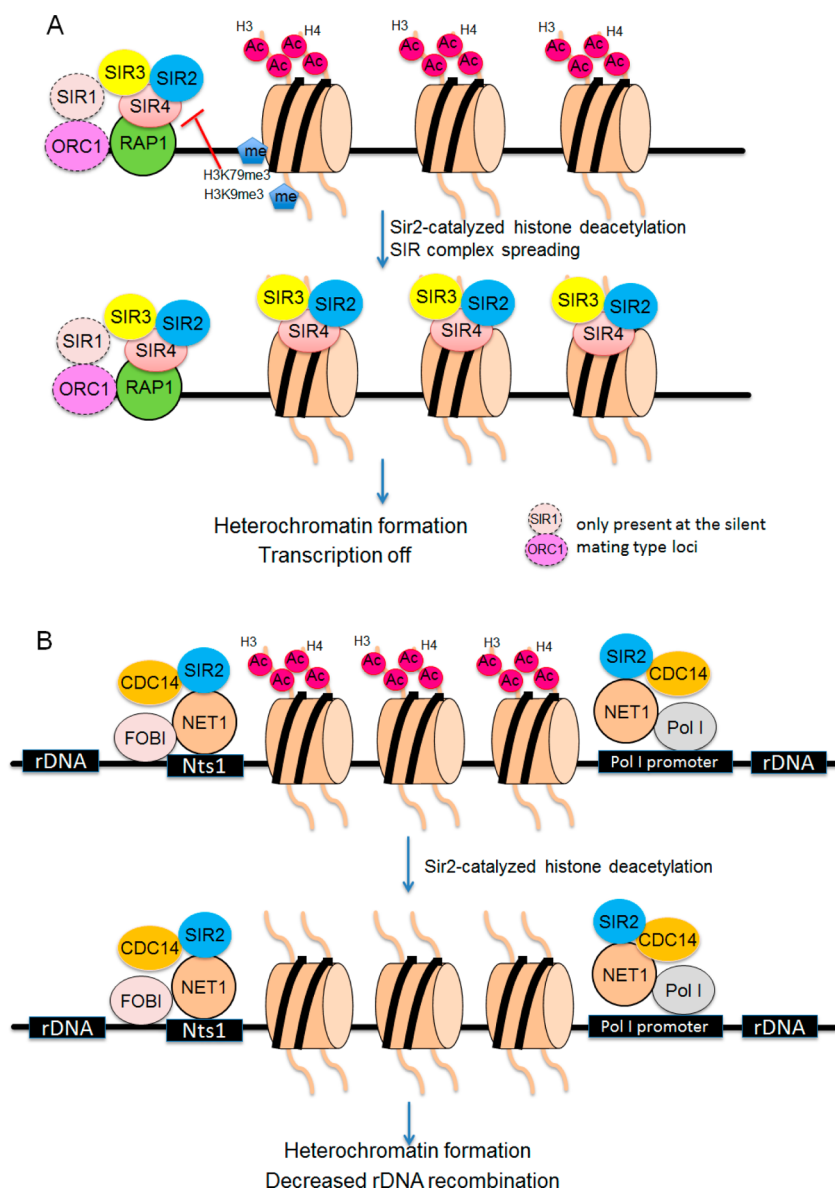
Figure 2. Models for the role of SIR2 in gene silencing in yeast. (A) SIR2 and SIR complex at the silent mating-type loci and telomeric heterochromatin. (B) SIR2 at silenced rDNA loci.

Mammalian SIRT4 is in class II, SIRT5 is in class III, while SIRT6 and SIRT7 are both in class IV.

The enzymatic reaction mechanism for sirtuin-catalyzed NAD-dependent protein lysine deacetylation (Figure 1) has been well understood through a series of elegant biochemical and structural studies.[20,21] The conserved catalytic core of sirtuins consists of a zinc-binding domain and a Rossmann fold domain (Figure 1C).[22−24] The active site lies at the interface of the two domains. It was thought that the acetyl lysine peptide binds first, followed by the binding of NAD.[25] Once the tertiary complex is formed, the carbonyl oxygen of the acetyl group attacks the C1′-position of the nicotinamide ribose, displacing nicotinamide and forming the alkylamidate intermediate (intermediate I, Figure 1B).[20] A conserved histidine residue then serves as a general base to deprotonate the ribose 2′-OH, which then attacks intermediate I at the carbonyl carbon, generating the 1′,2′-cyclic intermediate (intermediate II, Figure 1B). Intermediate II is then hydrolyzed to produce 2′-*O*-acetyl-ADP-ribose (2′-O-Ac-ADPR), which can be nonenzymatically isomerized to 3′-O-Ac-ADPR.[20,21] Using mechanism-based inhibitors (thioacyl-lysine peptides), an *S*-alkylamidate intermediate similar to intermediate I was captured in *Thermotoga maritima* SIR2 (TmSIR2) and human SIRT3 crystal structures,[26,27] and an intermediate similar to intermediate II was captured in SIRT5 crystal structure.[28]

Among the seven mammalian sirtuins, only the class I members (SIRT1−3) have shown robust deacetylase activity in vitro. SIRT4−7, in contrast, have very weak deacetylase activity in vitro.[29,30] It has been proposed that some of them may function as ADP-ribosyltransferases.[30,31] However, this activity is also very weak in vitro, and its physiological significance is still under debate.[32,33] Recently, it was demonstrated that mammalian SIRT5, one of the three mitochondrial sirtuins in mammals, functions to remove negatively charged acyl groups, such as succinyl and malonyl, from protein lysine residues (Figure 1A).[34] Protein lysine succinylation and malonylation were not previously known as common protein post-translational modifications (PTMs). These initial studies pointed out

that they are widely occurring PTMs in both bacterial and mammalian cells. Recent proteomic studies have identified close to a thousand proteins that are succinylated.[35,36] Many substrate proteins of SIRT5 are metabolic enzymes, and interestingly, SIRT5 can either activate or inhibit the substrate enzymes, depending on the enzymes being modified.[35] These studies also suggest that any abundant acyl-CoA molecule may be used to modify proteins.[37] Indeed, protein lysine glutarylation has recently been reported, and SIRT5 can also remove glutaryl group from proteins.[38] Similarly, protein lysine crotonoylation was also reported.[39]

SIRT5 recognizes the negatively charged acyl groups using an Arg residue and a Tyr residue, which interact with the carboxylate group via electrostatics and hydrogen-bonding interactions.[34] The Arg and Tyr residues are conserved in most class III sirtuins, raising the possibility that all class III sirtuins with the conserved Arg and Tyr residues may have the ability to remove negatively charged acyl groups.[34] Indeed, *E. coli* COBB has been recently shown to efficiently hydrolyze succinyl lysine.[40] However, different from SIRT5, COBB also has efficient deacetylase activity in vitro, suggesting that certain sirtuins can be promiscuous and multifunctional.

SIRT6, which also has weak and sequence-specific deacetylase activity toward peptide substrates in vitro,[41−43] was shown to have more efficient activity in removing long chain fatty acyl groups, such as myristoyl and palmitoyl (Figure 1A).[44] This activity is similar to the previously reported activity for the *Plasmodium falciparum* SIR2 protein, PfSIR2A.[45] One physiological substrate for the defatty-acylation activity of SIRT6 is tumor necrosis factor α (TNFα), which was known to be myristoylated on K19 and K20.[44] SIRT6 promotes the secretion of TNFα by defatty-acylation of K19 and K20 of TNFα. Although SIRT6 is a poor deacetylase in vitro, the deacetylation of histone H3K9 and H3K56 by SIRT6 has been intimately linked to the physiological function of SIRT6 in genome maintenance,[43] metabolism,[46] and inflammation.[47] Recent studies showed that the deacetylase activity of SIRT6 could be activated by nucleosomal substrates[48] or free fatty acids.[49] These findings may partly explain the discrepancy between in vitro and in vivo studies on SIRT6 activities and also raise the possibility that SIRT6 acts as a metabolic sensor with multiple switchable functions. SIRT1−3 can also remove long chain fatty acyl groups in vitro.[49] However, the physiological relevance remains to be confirmed. Similar to SIRT6, SIRT1−3 may exhibit context-dependent specificity toward certain acylation on Lys residues.

The different activities of SIRT1−3 (class I, mainly deacetylation but can also remove long chain fatty acyl groups), SIRT5 (class III, desuccinylation, demalonylation, deglutarylation), and SIRT6 (class IV, more efficient at defatty-acylation but can also remove acetyl) demonstrate that different classes of sirtuins may have different acyl lysine substrate specificity. Thus, the classification of sirtuins based on sequence homology is informative of their biochemical functions, especially acyl group specificity.

1.3. Biological Functions of Sirtuins

Although sirtuins were initially studied in the context of gene silencing and aging, in mammalian cells, many other biological functions have been revealed.[50−52] The biological function of sirtuins depends on the substrate proteins they modify. Because mammalian sirtuins have a very diverse set of substrates, it is not surprising that they affect many different biological

processes. Sirtuins regulate many aspects of chromatin biology, such as transcription, recombination, and genome stability, by modifying histones, transcription factors, and epigenetic enzymes. Sirtuins also regulate metabolism by modifying a diverse set of metabolic enzymes, both in the cytosol and in the mitochondria. The defatty-acylation activity of SIRT6 on TNFα also revealed that sirtuins can regulate protein secretion and membrane trafficking. The diverse substrate proteins of sirtuins also dictate that sirtuins may be involved in various human diseases, such as cancer, neurodegeneration, diabetes, and other metabolic disorders.[50−52] Below, we will focus on the role of yeast and mammalian sirtuins in epigenetic regulation.

2. YEAST SIRTUINS IN EPIGENETIC REGULATION

2.1. Role of SIR2 in Regulating Transcriptional Silencing

SIR2-mediated silencing is achieved by recruitment of SIR2 to different chromatin locations. SIR2 recruitment to the silent mating loci and telomeres requires RAP1 (repressor activator protein 1), which binds to specific silencer DNA sequences (Figure 2A). RAP1 interacts with SIR4, which in turn recruits SIR2 and SIR3.[53] ORC1 (origin recognition complex 1), a subunit of the origin recognition complex, is responsible to recruit SIR1 at the silent mating-type loci and helps to stabilize the silencing complex.[54−56] Once recruited to the silent mating loci and telomeres, SIR2 is thought to deacetylate various histones (H2B, H3, and H4) on multiple sites[6,57] in nearby nucleosomes, which creates sites with higher binding affinity for SIR3,[58] leading to spreading of the silencing complex across the heterochromatin regions[59−61] (Figure 2A). A histone H4K16 acetyltransferase, SAS2 (something about silencing 2), functions to oppose the role of SIR2 to prevent the spreading of the SIR complex to euchromatin.[62,63] Paradoxically, a zinc-dependent histone deacetylase (HDAC), RPD3 (reduced potassium dependency 3), was also reported to be important for preventing the spreading of SIR complex.[64,65] One proposal was that RPD3 removes SIR2 acetyl lysine substrates, thus eliminating the production of O-Ac-ADPR, which promotes SIR complex spreading.[65,66] However, the role of O-Ac-ADPR in SIR complex spreading is debated.[67]

At the rDNA loci, SIR2 is recruited by NET1 (nucleolar-silencing establishing factor and telophase regulator 1).[68] NET1, SIR2, and CDC14 together form a complex termed RENT (regulator of nucleolar silencing and telophase exit).[68,69] RENT binds to rDNA in mainly two regions: in one of the nontranscribed spacers (Nts1) and around the Pol I promoter. Binding to Nts1 requires FOB1 (fork blocking less 1), while binding to Pol I promoter requires RNA polymerase I[70] (Figure 2B).

How do SIR2-catalyzed histone deacetylation and the SIR complex repress transcription? Initial studies pointed to a model that the SIR complex silences transcription by promoting the formation of a more protected chromatin structure that is not accessible to the transcription machinery.[7] However, later studies suggested that silenced chromatin is actually permissive to activator binding and preinitiation complex formation.[71,72] It was proposed that SIR proteins silence gene transcription by blocking the transition from transcription initiation to elongation.[73] It was further demonstrated that short cryptic RNA Polymerase II transcripts are produced at the silenced rDNA loci. These cryptic transcripts are terminated by the NRD1/SEN1 (nuclear pre-mRNA downregulation 1/splicing endonuclease 1) complex

and degraded by the exosome. Disruption of the NRD1/SEN1/exosome pathway leads to decreased gene silencing.[73] These studies point to a more complex model on how SIR2 and SIR complex regulate gene silencing. Interestingly, the involvement of RNA in gene silencing seems to be a general theme. In the fission yeast, which has a very different gene-silencing mechanism, noncoding RNA and the RNAi machinery are required for the formation of heterochromatin.[74]

2.2. Role of SIR2 in Regulating rDNA Recombination

SIR2, as a member of the RENT complex, was also found to be important to prevent recombination events at the rDNA loci.[75] Interestingly, available experimental evidence support a model that the SIR2's role in suppressing rDNA recombination is achieved through regulating the transcription of noncoding RNA under the control of bidirectional promoter called "E-pro". It is thought that bidirectional E-pro transcription eliminates cohesin occupancy at the rDNA loci. SIR2, by suppressing E-pro transcription, maintains cohesin attachment and thus suppresses rDNA recombination between sister chromatids.[76,77] SIR2-dependent silencing is not sufficient to inhibit recombination within the rDNA locus, and it was demonstrated that perinuclear rDNA positioning also plays an important role in rDNA stability.[78] The perinuclear rDNA positioning is achieved via interaction between the nucleolar Cohibin complex and two inner nuclear membrane proteins HEH1 (helix extension helix 1) and NUR1 (nuclear rim 1).[78]

2.3. Role of SIR2 in Regulating DNA Repair

The SIR proteins are also required for repair of double-strand DNA breaks.[79] Deletion of *Sir2−4* impaired the ability of yeast cells to repair double-strand DNA breaks. Furthermore, the introduction of DNA double-strand breaks led to the relocalization of the SIR complex from telomeres to DNA double-strand breaks, which further led to derepression of telomeric genes.[80,81] Similarly, mammalian SIRT1 was also found to be important for repairing DNA double-strand breaks and undergo DNA damage-induced relocalization.[81] It was proposed that the DNA damage-induced SIRT1 relocalization and the corresponding gene expression change may contribute to aging.[81] This proposal is in line with the "heterochromatin island" hypothesis of aging proposed earlier.[82] A similar age-associated depression of silenced genes was also reported in flies.[83]

SIR complex relocalization was also observed when the yeast telomeres and silent mating-type loci clustering were disrupted.[84] Yeast telomeres and silent mating-type loci are clustered and form foci in the nuclear envelope. The SIR complex localizes to these foci. When the clustering was disrupted by deletion of yeast DNA repair factor KU70 and ESC1 (establishes silent chromatin 1), the SIR complex was released from the foci, which consequently changed gene transcription (derepression of subtelomeric genes and repression of genes in other locations).[84]

These studies suggest that SIR proteins may exist in limiting concentrations and preferentially occupy high-affinity binding sites on chromatin. However, under conditions that disrupt this normal localization or under conditions that create higher affinity binding sites, the SIR complex can relocalize to other places on the chromatin and lead to repression or derepression of transcription.

What is the role of histone acetylation/deacetylation in DNA double-strand break repair? It has been well documented that histone acetylation is required for the repair of DNA double-

strand breaks.[85−87] It has been shown that localized acetylation and deacetylation occur during homologous recombination to repair DNA double-strand break introduced by the HO endonuclease.[88] Several acetyltransferases (GCN5 and ESA1) and deacetylases (RPD3, SIR2, HST1) were recruited to double-strand breaks, and deletion of GCN5 and RPD3 led to inviability after induction of HO endonuclease. Consistent with this, it was observed that chromatin containing DNA double-strand breaks underwent local expansion and decondensation.[89] The chromatin decondensation may allow DNA repair factors to access the damaged DNA. In *Drosphila*, the acetylation of phosphorylated H2Av by TIP60 (60 kDa Tat-interactive protein) is thought to promote the exchange of phosphorylated H2Av by unphosphorylated H2Av following DNA repair, which serves as a mechanism to indirectly dephosphorylate H2Av at the damage site to complete DNA repair.[90] In mammals, the histone acetyltransferase HAT1 was also reported to function in DNA double-strand repair by promoting histone turnover.[91] Whether such a mechanism also applies in yeast and other eukaryotes is not clear. However, histone H3K56 acetylation, catalyzed by RTT109 and histone chaperon ASF1, has been shown to be important for reassembly of nucleosomes after DNA double-strand break is repaired.[92] Thus, even though a detailed picture is still unavailable, progress toward such a picture is emerging.

2.4. Interaction between SIR2 and Other Epigenetic Markers/Enzymes

2.4.1. SIR2 Interacts with Other Proteins That Control Histone Acetylation.
As discussed before, the histone H4K16 acetyltransferase, SAS2, functions to oppose the role of SIR2 to prevent the spreading of the SIR complex to euchromatin.[62,63] BDF1 (bromodomains factor 1) bromodomain binds to acetylated histone H3 and H4 and competes with SIR2. This is thought to be important in maintaining a transcriptional active euchromatin.[93] These observations are easy to understand as histone acetylation and deacetylation should have opposite effect.

The more puzzling observation was that a zinc-dependent HDAC, RPD3, was important for preventing the spreading of SIR complex.[64,65] One proposal was that RPD3 removes SIR2 acetyl lysine substrates, thus eliminating the production of O-Ac-ADPR, which promotes SIR complex spreading.[65,66] However, the role of O-Ac-ADPR in SIR complex spreading is debated.[67] Similarly, ESA1, a MYST family acetyltransferase that is generally associated with transcription activation and double-strand DNA repair, is also reported to be important for silencing at the rDNA loci, similar to SIR2.[94] Therefore, mechanistically, more needs to be further elucidated.

2.4.2. SIR2 and Histone Methylation.
SIR2-dependent gene silencing is regulated by the protein Arg methyltransferase HMT1 (HnRNP methyltransferase 1) in yeast. Lack of HMT1 activity leads to decreased histone H4R3 methylation, increased histone acetylation, and increased transcription from silent chromatin regions and increased mitotic rDNA recombination.[95]

H3R2 methylation is reported to be required for gene silencing at the same sites that are silenced by SIR2.[96] H3R2 methylation suppresses H3K4 trimethylation and transcription. However, H3R2 methylation does not affect SIR2 recruitment to these sites. Thus, it is not clear how H3R2 methylation promotes gene silencing. It would be interesting to see whether

H3R2 methylation regulates the histone H3 or H4 acetylation level.

SIR2 occupancy at the silenced rDNA loci decreased when K79 was mutated to Ala/Pro/Gln.[97] At the silent mating loci, SIR3 occupancy and silencing increased when DOT1 (disruptor of telomeric silencing 1, methylates H3K79) and SET1 (SET domain-containing 1, methylates H3K4) were deleted.[98] When H3 K4/K79 were mutated to Arg, the SIR complex recruitment increased and inhibited the nucleoside excision repair at heterochromatin.[99] In vivo, H3 K79 is hypomethylated in silent loci.[100] In vitro, DOT1-mediated H3K79 methylation decreases SIR complex binding.[101] These evidence suggest that the SIR complex prefers the unmethylated H3K4 and H3K79 (or the K to R mutation, which mimics the unmethylated K), while methylation or other mutations will decrease SIR complex binding. However, the situation was complicated by reports that deletion of *Dot1* decreased telomeric silencing.[97,102] It has been proposed that H3K4 and H3K79 methylation in euchromatin help to prevent nonspecific binding of the SIR complex, thus promoting silencing at heterochromatin. When *Dot1* or *Set1* is deleted, SIR complex may have increased nonspecific binding to euchromatin and thus decrease silencing at heterochromatin.[27,103] Recently, it was reported that the complication might be due to an artifact caused by the use of the *Ura3* reporter gene to read out gene-silencing effects at specific telomeric positions and DOT1's effects on gene silencing at heterochromatin is rather limited and not general.[104,105]

2.5. Functions of Other Yeast Sirtuins in Epigenetic Regulation

Four SIR2 homologues (HST1−4) were found in yeast, and three of them, HST1, HST3, and HST4, were thought to have similar gene-silencing activity.[106] HST1 is the closest homologue of SIR2 and was thought to have distinct but partial overlapping functions.[107] Under certain genetic conditions, HST1 overexpression can restore repression that was lost due to *Sir2* deletion.[108] HST1 is important for the repression of middle sporulation-specific gene expression during mitosis.[109] It also represses genes involved in de novo NAD and thiamine biosyntheses.[107,110] HST1 is recruited to the promoters of the repressed genes by a sequence-specific transcriptional repressor SUM1, which recognizes DNA sequences called Mse (middle sporulation element), and a tethering factor called RFM1 (repression factor of middle sporulation element 1).[109,111] The SUM1-RFM1-HST1 complex is also thought to be important for replication initiation at the origin of replication via histone H4K5 deacetylation.[112] However, the detailed mechanism is not clear.

HST2 is mainly cytosolic[113] but can shuttle between the nucleus and the cytoplasma.[114] Not much is known about its physiological role. However, overexpression of HST2 can partially suppress the silencing defects in *Sir2* deletion.[113] It has also been reported that SIR2-independent life span extension is through the silencing effect of HST2.[115] However, this is controversial.[116]

HST3 and HST4 have been reported to deacetylate histone H3K56 in a cell cycle-dependent manner.[117,118] Both deletion and overexpression of HST3/HST4 increased the sensitivity of yeast cells to genotoxic reagents that interfere with replication fork progression.[118] During S phase, virtually all newly synthesized histone H3 is acetylated on K56[117] and are incorporated into newly formed nucleosome. Furthermore,

both the lack of deacetylation and the lack of acetylation of on H3K56 lead to hypersensitivity to genotoxic reagents,[117,118] but the exact molecular mechanism is not clear.[119] HST3 and HST4 have also been reported to contribute to gene silencing. HST3 is required for silencing the 2mu replication origin.[120] Deletion of both *Hst3* and *Hst4* leads to a defect in telomeric-silencing defects, which can be rescued by deletion of the H3K56 acetyltransferase gene *Rtt109*.[121]

3. MAMMALIAN SIRTUINS IN EPIGENETIC REGULATION

Among the seven mammalian sirtuins, SIRT1, SIRT2, SIRT6, and SIRT7 have all been demonstrated to have important epigenetic roles. In contrast, SIRT3−5 are considered to localize mainly in the mitochondria, where they regulate numerous mitochondrial proteins. Therefore, the roles of SIRT3−5 are mainly in metabolic regulation. However, several reports suggested the role of SIRT3 in epigenetic regulation,[122−124] although it remains controversial.[125] Similarly, SIRT5 also have nonmitochondrial desuccinylation substrates,[35,36,126] and histones can be succinylated,[127] but it is not clear whether SIRT5 can catalyze histone desuccinylation. Therefore, knowledge about the epigenetic roles of SIRT3−5 is very limited at this point, and we will focus on the epigenetic roles of SIRT1, SIRT2, SIRT6, and SIRT7 here.

3.1. Epigenetic Functions of SIRT1

SIRT1 is by far the most understood mammalian sirtuin and has the greatest homology to yeast SIR2. SIRT1 is predominantly present in the nucleus,[29] yet it shuttles between the nucleus and the cytoplasm in a context-dependent manner. SIRT1 deacetylates lysine residues in both histones and nonhistone proteins, thereby regulating transcription, protein stabilities, and activities. SIRT1 controls various cellular processes (Table 1), such as chromatin organization, metabolism, cell survival, differentiation, and development, as well as stress responses. Although the role of SIRT1 is far more expansive (Table 1), the majority of its functions are intimately connected to epigenetic regulation. The role of SIRT1 in epigenetics is achieved via several different mechanisms, such as regulating chromatin structure by histone deacetylation, regulating the activity of transcription factors by deacetylation, and regulating the activity of other epigenetic enzymes by deacetylation (Figure 3).

3.1.1. SIRT1's Histone Deacetylase Activity Regulates Chromatin Structure and Transcription. SIRT1 is involved in the formation of both facultative and constitutive heterochromatin. Like other sirtuins, SIRT1 is not capable to directly bind to DNA but needs to be recruited by a variety of chromatin-associated factors to their binding sites. Due to its remarkable ability to bind to many factors, SIRT1 acts as a coordinator of heterochromatin formation instead of being merely a histone deacetylase. Recruitment of SIRT1 to chromatin is usually associated with the epigenetic silencing of target genes and heterochromatin formation.

SIRT1 was shown to deacetylate lysine residues of the N-terminal tails of H3 and H4, preferentially H4K16, and to a less extent H3K9. The deacetylation of histone residues H3K14, H4K8, and H4K12 by SIRT1 was also observed in a biochemical assay in vitro, but it was shown to occur at a slower rate.[14,128] RNAi-mediated decrease in SIRT1 expression in human cells led to hyperacetylation of H3K9 and H4K16, together with reduction in the repressive chromatin marks,

Table 1. SIRT1 Deacetylation Substrates

substrate	full name	site of modifications	function of SIRT1-catalyzed deacetylation
histones			
H1	histone H1	K26	promote the formation of heterochromatin and transcriptional repression[81,128]
H3	histone H3	K9, K14, K56	deacetylation of H3K9 and H3K14 promotes transcriptional repression and heterochromatin formation;[14,128] deacetylation of H3K56 helps maintain genome stability[150,151] and also inhibit transcription of Bcaf1 in T cells[152]
H4	histone H4	K16	promote the formation of heterochromatin and transcriptional repression[14,81,128]
chromatin modifying enzyme and structural proteins			
DNMT1	DNA (cytosine-5)-methyltransferase 1	K160, K188, K259, K366, K749, K891, K957, K961, K975, K1054, K1111, K1113, K1115, K1117, K1349, K1415	deacetylation of K1349 and K1415 in the catalytic domain increases DNMT1 activity; deacetylation of lysine residues in the GK linker decreases DNMT1's methyltransferase-independent transcriptional repression function; deacetylation of all identified acetylated lysine sites in DNMT1 abrogates its binding to SIRT1 and impairs its capability to regulate cell cycle G2/M transition[174]
HDAC1	histone deacetylase 1	K89, K220, K432, K438, K439, K441	stimulate HDAC1 activity after DNA double-strand break (DSB) induction, which is critical for DSB repair by the nonhomologous end-joining pathway[207]
hMOF	human ortholog of MOF	K274	increase the chromatin recruitment of hMOF to Hoxa9 promoter and the H4K16 acetylation level in Hela cells[158] or inhibit the acetyltransferase activity and promote its ubiquitination-dependent degradation[159]
P300		K1020, K1024	repress its transactivation[156] and destabilize it by promoting ubiquitination[157]
PCAF	histone acetyltransferase KAT2B	unknown	repress its transactivation and retard muscle differentiation in response to redox stress[140]
SATB1	DNA-binding protein SATB1	K136, K175	facilitate the inter-MAR (matrix attachment region) association and to promote ε-globin gene expression[208]
SUV39H1	suppressor of variegation 3–9 homologue 1	K266	upregulate the methyltransferase activity of SUV39H1[163] and stabilize SUV39H1,[163] thereby promoting methylation of H3K9
TIP5	transcription termination factor I-interacting protein 5	K633	promote the binding of NoRC with promoter RNA (pRNA) and increase the heterochromatin histone marks at rDNA loci[175]
TIP60	60 kDa Tat-interactive protein	K327	inhibit the acetyltransferase activity and protein stability of TIP60[159,161]
transcription factors			
AR	androgen receptor	K630, K632, K633	repress dihydrotestosterone (DHT)-induced AR signaling by inhibiting coactivator-mediated interaction between the AR N- and C-termini[209]
BMAL1	brain and muscle ARNT-like 1	K537	keep CRY from binding to BMAL1 and facilitate transactivation of CLOCK/BMAL1[204]
c-JUN	proto-oncoprotein c-JUN	K268, K271, K273	inhibit the activity of transcription factor AP-1, leading to T-cell anergy and diminished T-cell activation[210]
c-MYC	proto-oncoprotein c-MYC	K323	facilitate c-MYC/MAX interaction and stabilize c-MYC, leading to increased c-MYC transactivation activity,[199,211] or destabilized c-MYC[212]
CIITA	class II transactivator	unknown	shield CIITA from proteasomal degradation and promote its nuclear accumulation and transactivation on MHC II (major histocompatibility complex II) during antigen-dependent T-cell stimulation[213]
CRTC1	CREB-regulated transcription co-activator 1	K13, K20, K33, K178, K197	activate CRTC1 by facilitating its dephosphorylation and interaction with CREB, thus activating the transcriptional networks in both the normal and the Huntington's disease brain[214]
CRTC2	CREB regulated transcription co-activator 2	K628	destabilize CRTC2, resulting in decreased CRTC2/CREB-mediated gluconeogenesis during long-term fasting[181]
DCOH2	dimerization cofactor of HNF-1α	unknown	promotes its dimerization with hepatocyte nuclear factor 1alpha (HNF-1α), leading to increased DNA binding of HNF-1α and intestinal farnesoid X receptor (FXR) signaling, and subsequent alteration of systemic bile acid homeostasis[215]
E2F1	E2F transcription factor 1	K117, K120, K125	inhibit E2F1 transcriptional and apoptotic activity in response to DNA damage[216]
ERα	estrogen receptor α	K266, K268	increase[217] or decrease[218] its DNA-binding affinity and transactivation
FOXA2	forkhead box protein A2	K6, K259, K264, K273, K275	target FOXA2 toward proteasomal degradation and inhibit FOXA2-mediated fatty acid oxidation and ketogenesis during fasting;[219,220] promote the transcription of Pdx1 (pancreas duodenum homeobox 1) and β-cell formation[221]
FOXO1	forkhead box protein O1	K242, K245, K262	promote FOXO1-mediated transcription during gluconeogenesis,[181] adipogenesis,[222] lipolysis,[223] starvation-induced autophagy,[224] response to oxidative stress[225] and nitric oxide,[226] muscle growth[227] and apoptosis;[228] restrain the antiangiogenic activity of FOXO1 and promote vascular growth[229]
FOXO3a	forkhead box protein O3a	unknown	inhibit its transcription activation and ability to induce apoptosis[186,230] but increase its ability to induce cell cycle arrest and resistance to oxidative stress[186]
FOXO4	forkhead box protein O4	unknown	activate FOXO4-dependent transcription of stress-regulating genes[231,232]
FOXP3	forkhead box protein P3	unknown	lead to FOXP3 polyubiquitination and proteosome-mediated degradation and decreased numbers of regulatory T cells[193–196]
FXR	farnesoid X receptor	K217	decrease its stability but promote heterodimerization with RXRα, DNA binding, and transactivation activity,[233] promote FXR-mediated hepatitis B virus transcription[234] and bile acid homeostasis during liver regeneration[235]

Table 1. continued

substrate	full name	site of modifications	function of SIRT1-catalyzed deacetylation
transcription factors			
HIF-1α	hypoxia-inducible factor 1α	K674	repress the transactivation of HIF-1α during hypoxia[197]
HIF-2α	hypoxia-inducible factor 2α	K385, K685, K741	activate HIF-2α, thereby promoting HIF-2 signaling during hypoxia[198]
HSF-1	heat shock factor 1	K80	prolong HSF-1 binding to the heat shock promoter;[236] promote HSF-1-mediated transcription of HSP70 in response to alpha-synuclein aggregation-induced stress[237] and repression of IGF-IIR expression and cardiomyocyte apoptosis[238]
LXRα	liver X receptor protein α	K432	activate LXRα, leading to increased expression of various LXR targets involved in lipid metabolism[239]
MeCP2	methyl-CpG binding protein 2	K464	deacetylation keeps it from binding to the brain-derived neurotrophic factor (BDNF) promoter in hippocampi[240]
MyoD	myogenic determining factor	K99, K102, K104	repress its transactivation and retard muscle differentiation in response to redox stress[140]
NFAT	nuclear factor of activated T cells	unknown	suppress the transcriptional activity of NFAT, leading to the inhibition of PMA/Ionomycin-induced expression of COX-2[241]
NHLH2	helix–loop–helix protein 2	K49	SIRT1 deacetylates NHLH2 to activate the monoamine oxidase A (MAO-A) promoter, thereby decreasing serotonin levels in the brain[242]
NOTCH	neurogenic locus notch homologue protein 1	K1764, K1770, K1771, K1772, K1785, K1935, K2050, K2068, K2146, K2147, K2150, K2154, K2161, K2164	destabilize NOTCH, thereby limiting the DDL4/NOTCH signaling in endothelial cells[243]
NPM1	nucleophosmin 1	K212, K215, K229, K230, K257, K267	reduce its activity to promote transcription of genes implicated in oral cancer[244]
p53	tumor suppressor p53	K382	negatively regulate transactivation activity of p53, thereby attenuating p53-dependent apoptosis induced by DNA damage and oxidative stress,[188] as well as PML/p53-induced cellular senescence[189]
p73	tumor protein p73	unknown	suppress p73-dependent transactivation[245]
PER2	period circadian protein homologue 2	unknown	promote the degradation of PER2, thereby derepressing CLOCK/BMAL1-mediated expression of circadian clock gene expression[203]
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α	K77, K144, K183, K253, K277, K270, K320, K346, K412, K441, K450, K757, K778	stimulate its transcription coactivator activity on mediating gluconeogenesis,[181,182,246] fatty acid oxidation,[184] mitochondrial biogenesis,[247,248] and expression of BMAL1 and CLOCK;[249] augment PPARγ-PGC-1α-mediated repression of beta-secretase/BACE1;[250] promote hepatitis B virus transcription[234]
PTF1A	pancreatic transcription factor-1a	unknown	SIRT1 colocalizes with PTF1A at the acinar gene promoters and promotes acinar-to-ductal metaplasia[251]
RARβ	retinoic acid receptor-β	unknown	activate RARβ and promote the transcription of the α-secretase gene ADAM10, thereby inhibiting β-amyloid production[252]
RelA/p65	RelA/p65 subunit of nuclear factor-κB	K310	repress NF-κB-dependent transcription, augmenting TNFα-induced apoptosis[192]
RFX5	regulatory factor for X-box RFX5	unknown	promote its nuclear exclusion and proteasomal degradation, thereby derepressing collagen type I (COL1A2) transcription by RFX5 in smooth muscle cells[253]
SMAD3	mothers against decapentaplegic homologue 3	unknown	repress the transactivation of SMAD3 following TGF-β1 in a chronic kidney disease (CKD) model[254]
SMAD4	mothers against decapentaplegic homologue 4	unknown	repress the effect of TGF-β signaling on MMP7 and therefore the EMT transition in cancer metastasis[255]
SMAD7	mothers against decapentaplegic homologue 7	K64, K70	promote SMAD ubiquitination regulatory factor 1 (Smurf1)-mediated proteasome degradation and TGF-β-induced apoptosis in glomerular mesangial cells[256]
SREBP-1c	sterol regulatory element-binding protein 1c	K289, K309	inhibits its transactivation by destabilizing it and reducing its binding affinity for promoters of lipogenic target genes[185,257]
STAT3	signal transducer and activator of transcription 3	K679, K685, K707, K709	repress the inhibitory effect of STAT3 on gluconeogenic gene expression during long-term fasting[183]
STAT5	signal transducer and activator of transcription 5	K681, K694, K701, K705	negatively regulate GH-induced STAT5 phosphorylation and IGF-I production during fasting in the liver[258]
TAF₁68	TATA box-binding protein-associated factor RNA polymerase I subunit B	unknown	decrease its DNA-binding activity and repress RNA polymerase I transcription[259]
TAT	human immunodeficiency virus (HIV) TAT protein	K50	SIRT1 deacetylates TAT and acts as a transcriptional coactivator during Tat-mediated transactivation of HIV long terminal repeat[260,261]
YAP2	mammalian Ste20-like kinase/Yes-associated protein 2	K76, K90, K97, K102	increase the YAP2/TEAD4 (TEA Domain Family Member 4) association in hepatocellular carcinoma (HCC) cells, resulting in YAP2/TEAD4 transcriptional activation and increase in cell growth[262]

Table 1. continued

substrate	full name	site of modifications	function of SIRT1-catalyzed deacetylation
transcription factors			
β-catenin	β-catenin	K49, K345	promote its translocation from nucleus to cytoplasm and destabilize it, limiting its ability to activate transcription and drive cell proliferation;[251,263,264] restore nuclear localization of β-catenin in Mesenchymal stem cells (MSCs) and promote gene transcription for MSC differentiation[265]
DNA damage repair-related substrates			
APE1	apurinic/apyrimidinic endonuclease-1	K6, K7	promote base excision repair of damaged DN.[266]
KU70	DNA repair factor KU70	K539, K542	cause it to sequester the pro-apoptotic factor Bax (BCL2-Associated X Protein) away from mitochondria, thus inhibiting stress-induced apoptotic cell death;[267] promotes its DNA repair activity[268]
MCM10	protein MCM10 homologue	K312, K390, K683, K745, K761, K768, K681 + K682, K737 + K739, K847 + K849, K868 + K874, K683 + K685, K674 + K682	modulate its stability and ability to bind DNA; promote its function in DNA replication fork initiation[269]
NBS1	Nijmegen Breakage Syndrome 1	unknown	deacetylation of NBS1 is required for ionizing radiation-induced NBS1 phosphorylation at Ser343, which is essential for the activation of S phase checkpoint and for efficient DNA damage repair response[270]
WRN	Werner syndrome ATP-dependent helicase	unknown	promote its helicase and exonuclease activities and facilitate its translocation from nucleoplasm to nuclei in response to DNA damage[271]
XPA	xeroderma pigmentosum group A	K63, K67	SIRT1-mediated deacetylation of XPA is essential for optimal nucleotide excision repair (NER) pathway during UV-induced DNA repair[272]
other substrates			
AceCS1	acetyl-CoA synthetase 1	K661	activate AceCS1 to convert acetic acid to acetyl-CoA for use in fatty acid synthesis[273]
AKT	RAC-alpha serine/threonine-protein kinase	unknown	activate AKT and promote axonogenesis[274]
ATG5	autophagy-related protein 5	unknown	promote the induction of autophagy[275]
ATG7	autophagy-related protein 7	unknown	promote the induction of autophagy[275]
ATG8	autophagy-related protein 8	unknown	promote the induction of autophagy[275]
BCL6	B-cell lymphoma 6 protein	K379	activate its oncogenic activit.[276]
cortactin	cortactin	unknown	facilitate cancer cell migration[277]
CREB	cAMP response element binding protein	K136	repress its activity by preventing its phosphorylation, which leads to decreased expression of gluconeogenic genes and increased hepatic lipid accumulation and secretion[278]
eNOS	endothelial nitric oxide synthase	K496, K506	activate eNOS in the cytoplasm, which increases NO level, leading to vasodilatation, increased blood flow, and nutrient delivery to tissues[279]
EVI1	ectopic viral integration site I	unknown	lead to destabilization of the protein[280]
HMGB1	high-mobility group box 1	K55, K88, K90, and K177	repression of SIRT1 induced by inflammation disables deacetylation of HMBG1 and promotes the nuclear-to-cytoplasmic translocation and release into circulation, thereby maintaining inflammation[281]
HMGCS1	hydroxymethylglutaryl-CoA synthase 1	unknown	unknown[282]
IRS-1	insulin receptor substrate 1	unknown	promote systemic insulin resistance in neurons[283]
IRS-2	insulin receptor substrate 2	unknown	promote its phosphorylation and activate the IGF-1/Ras/ERK1/2 pathway, sensitizing neurons to oxidative damage[284]
LIN28	protein LIN-28	unknown	restore its stability[285]
MMP2	matrix metalloproteinase-2	unknown	stabilize MMP2, thereby increasing tumor cell invasion in prostate cancer[286]
PARP1	poly(ADP-ribose) polymerase 1	K498, K505, K508, KS21, KS24	attenuate PARP1 activity and protect cells from PARP1-dependent cell death under stress conditions[287]
PGAM1	glycolytic enzyme phosphoglycerate mutase-1	K251, K253, K254	decrease PGAM1 activity in glycolysis[288]
PIP5Kγ	phosphatidylinositol-4-phosphate 5-kinase gamma	K265, K268	activate PIP5Kγ and promote the secretion of thyroid stimulating hormone (TSH) from pituitary cells[289]
PTEN	phosphatase and tensin homologue	unknown	SIRT1 deacetylates PTEN to inhibit AKT and trigger apoptosis under antioxidant-free conditions[290]
RB	retinoblastoma tumor suppressor protein	K873, K874	allow phosphorylation of RB and relieve RB-mediated repression of E2F-regulated cell cycle genes[291]

Table 1. continued

substrate	full name	site of modifications	function of SIRT1-catalyzed deacetylation
other substrates			
S6K1	p70 ribosomal S6 kinase	K484, K485, K493	decrease the Thr-389 phosphorylation and kinase activity of S6K1[292]
Tau	microtubule-associated protein tau	unknown	destabilize tau protein, resulting in the decrease in tau protein aggregates[293]
TDG	thymine DNA glycosylase	unknown	increase TDG glycosylase activity and weakly shift its activity toward T/G, 5-formylcytosine/G, and 5-carboxylcytosine/G compared from 5-fluorouracil/G[294]
TIAM1	T-cell lymphoma invasion and metastasis 1	K1420	promotes activation of DVL/TIAM1/Rac axis and cell migration in cancer cells[295]
zyxin	zyxin	unknown	SIRT1 deacetylates Zyxin and modulates its activity upon treatment with leptomycin B[296]
14-3-3ζ	14-3-3ζ	K49, K157, K212	keep 14-3-3ζ from dissociating from caspase-2, thereby antagonizing caspase-2-dependent apoptosis[297]

H3K9me3 and H4K20me1, suggesting that SIRT1 promotes facultative heterochromatin (FH) formation.[128] Further study showed that SIRT1 interacts with and deacetylates the linker histone H1 at Lys26 (H1K26). Localizing SIRT1 to the promoter of a Gal4-reproter integrated to euchromatin led to deacetylation of H3K9 and H4K16, recruitment of H1, loss of H3K79me2 (a mark associated with active transcription), and subsequent dramatic decrease in the expression of the reporter gene.[128] Notably, arrival of SIRT1 to chromatin results in spreading of hypomethylated H3K79, indicating the role of SIRT1 as a coordinator of heterochromatin formation.

Many functions of SIRT1 depend on its ability to deacetylate H3K9 and H4K16 and to mediate subsequent transcription repression. The most important mechanism by which SIRT1 promotes cellular response to stress is the transcription silencing associated with FH formation. Growing evidence illustrates the role of SIRT1 in metabolism, cell differentiation and development, cancer, and other diseases through forming corepressor complexes with numerous transcription repressors.

For example, genome-wide study by Oberdoerffer et al. showed that SIRT1 represses a large variety of genes across the mouse genome.[81] Upon oxidative stress, SIRT1 redistributes on chromatin and localizes to DNA strand break repair sites. The relocation of SIRT1 promotes DNA repair and also results in epigenetic changes surrounding the break sites. Redistribution of SIRT1 leads to global changes in the H1K26ac pattern and transcription. Notably, the changes in transcription of SIRT1-bound genes parallel those in aging mouse brain, suggesting the role of SIRT1 in maintaining genome stability and protecting cells from aging.[81] Ghosh et al. reported that SIRT1 inhibits NF-κB (nuclear factor κB)-mediated transcription by interacting with TLE1 (transducing-like enhancer of split-1), which is a non-DNA binding corepressor for NF-κB.[129]

Regarding the regulation of metabolism, SIRT1 stimulates the secretion of insulin in response to glucose through repressing the expression of UCP2 (uncoupling protein 2) in pancreatic β-cells. SIRT1 is recruited to *Ucp2* promoter and inhibits its transcription.[130,131] Yet how SIRT1 is recruited remains unclear. These findings suggest that a relationship between loss of SIRT1 activity and age-related type 2 diabetes.[132] SIRT1-containing corepressor complexes also target various nuclear receptors. One important example is the ERRs (estrogen receptor-related receptors), which regulate genes involved in mitochondria function and energy homeostasis.[133] SIRT1 inhibits the transactivation of ERRγ by forming a complex with the transcription corepressor of ERRγ at the ERRE (ERR-responsive element). It was further demonstrated that the repression of ERRγ target genes is dependent on SIRT1 activity to deacetylate H3K9 at ERRE.[134] In cardiac myocytes, PPARα (peroxisome proliferator-activated receptor α) binds and recruits SIRT1 to ERRE to inhibit ERR target genes involved in mitochondrial respiration, thereby mediating cardiac hypertrophy.[135,136] In white adipose tissue, SIRT1 promotes fat mobilization by suppressing genes controlled by PPARγ. During fasting, SIRT1 interacts with the corepressors of PPARγ, NCoR (nuclear receptor corepressor), and SMRT (silencing mediator of retinoid and thyroid hormone receptors), leading to decreased PPARγ transactivation and adipogenesis.[137] In hepatocytes, SIRT1 is recruited to liver receptor homologue-1 (LRH1) target gene promoters by orphan nuclear receptor SHP (small heterodimer partner), a transcriptional corepressor of various nuclear receptors. The recruitment of SIRT1 leads to histone H3/H4 deacetylation
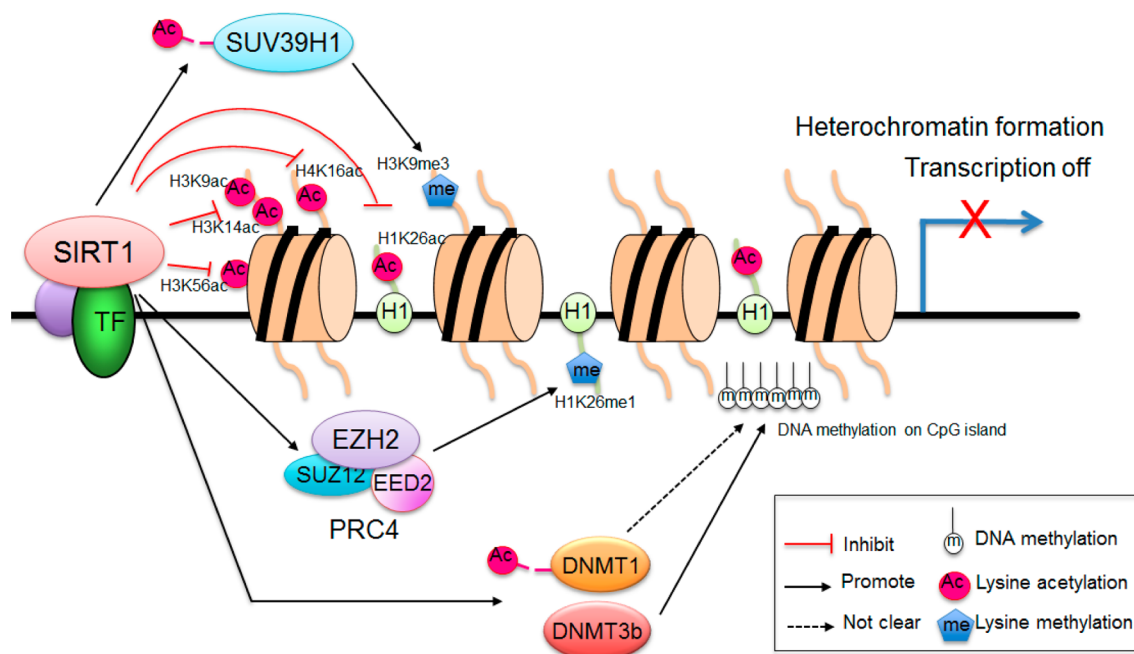
Figure 3. Model for SIRT1-mediated heterochromatin formation and transcriptional silencing.

and inhibition of LRH1-dependent *Cyp7a1* (*cholesterol 7α hydroxylase*) gene transcription, thereby regulating LRH1-dependent bile-acid homeostasis.[138] Moreover, screening for transcription factors that interact with SIRT1 in response to nutrient restriction identified HNF-1α (hepatocyte nuclear factor 1-α), a transcription factor that regulates the expression of several liver-specific genes. Formation of HNF-1α-SIRT1 complex on the *CRP* (*C-reactive protein*) gene promoter results in deacetylation of H4K16 and subsequent inhibition of HNF-1α-mediated transcription of *Crp* gene.[139]

Accumulating evidence shows that SIRT1-mediated chromatin silencing also has critical roles in development. For instance, SIRT1 inhibits myogenesis in response to redox stress by forming complex with PCAF/MyoD and deacetylating H3K9/H3K14 at the promoters of myogenin and MHC (myosin heavy chain).[140] SIRT1 directly interacts with BCL11 (B-cell leukemia 11) proteins, which are implicated in hematopoietic cell development and malignancies. Recruitment of SIRT1 by BCL11 increases deacetylation of H3/H4 and promotes transcription repression induced by BCL11 proteins.[141,142] SIRT1 also plays a role in hematopoietic stem and progenitor cell maintenance. SIRT1 counteracts the upregulation of HOXA9 (Homeobox protein Hox-A9) in response to hematopoietic stress by binding to the HOXA9 locus and deacetylating H4K16, thereby blocking the expansion of hematopoietic stem and progenitor cells.[143] SIRT1 is also involved in redox-dependent differentiation of neural progenitor cells by inhibiting the expression of pro-neuronal bHLH (basic helix−loop−helix) transcription factor MASH1 (mammalian achaete scute homologue 1). SIRT1 functions together with HES1 (hairy and enhancer of split-1) as transcriptional repressors. Under oxidative condition, SIRT1 is upregulated and recruited by HES1 to the *Mash1* promoter region, leading to the deacetylation of H3K9 but not H4K16, as well as the stabilization of the TLE1-containing repressor complex and subsequently to *Mash1* repression.[144] Additionally, BCL6 (B-cell lymphoma 6) promotes neurogenesis by excluding the coactivator MAML1 (Mastermind-like 1) and recruiting SIRT1

to the NOTCH-dependent transcriptional complex at the *Hes5* promoter. SIRT1-mediated epigenetic silencing of *Hes5* then leads to neuronal differentiation.[145]

Lastly, SIRT1-mediated FH formation and transcription silencing has also been implicated in various diseases, including neurodegeneration diseases and cancer. SIRT1 is involved in the *Fmr1* (*fragile X mental retardation 1*) gene silencing in fragile X mental retardation syndrome by deacetylating H3K9 and H4K16. Inhibition of SIRT1 reactivates Fragile X alleles in neurons.[146] SIRT1 also plays an important role during BRCA1-associated tumorigenesis. BRCA1 binds to SIRT1 promoter and promotes its expression, which in turn represses the oncogene *Survivin* by deacetylating histone H3. Hence, loss or mutation of *Brca1* leads to an increase in the expression of *Survivin* and subsequently cell proliferation and cell survival.[147] In prostate cancer cells, SIRT1 induces epithelial-to-mesenchymal transition (EMT) by suppressing E-cadherin expression. The EMT-inducing transcription factor ZEB1 (zinc finger E-box binding homeobox 1) recruits SIRT1 to the E-cadherin promoter, leading to H3K9 deacetylation and E-cadherin transcriptional suppression.[148]

Interestingly, SIRT1 could also serve as a transcriptional activator. The ING (inhibitor of growth) subunits of mSIN3A/HDAC1 complex can recruit SIRT1 to the HDAC-dependent transcriptional repression domain of RBP1 (retinoblastoma-binding protein 1). In addition, SIRT1 activity is able to negatively regulate RBP1-mediated transcription repression.[149]

SIRT1 has also been linked to DNA replication and DNA damage repair through deacetylation of H3K56. Acetylation of H3K56 was shown to play a critical role in assembling newly synthesized DNA into chromatin following DNA replication and DNA damage repair.[150,151] SIRT1 deacetylates H3K56 in vitro. Moreover, *Sirt1* knockdown or knockout mammalian cells display hyperacetylation of H3K56[150,151] and genomic instability.[151] Thus, it was proposed that deacetylation of H3K56 contributes to the role of SIRT1 in the regulation of genomic stability. In addition, SIRT1 also exerts its transcriptional repression role by deacetylating H3K56. SIRT1

deacetylates H3K56 at the promoter region of *Bclaf1* (*Bcl2-associated factor 1*) and represses its transcription, thereby negatively regulating T-cell activation.[152]

3.1.2. SIRT1 Regulates Other Epigenetic Enzymes.

The role of SIRT1 in transcriptional repression also involves interaction with and deacetylation of numerous epigenetic factors. One important aspect is that SIRT1 interacts with other histone-modifying enzymes and regulates their activities.

3.1.2.1. SIRT1 Regulates the Activity of Various Histone Acetyltransferases (HATs).

Various studies suggest that HATs and HDACs are interdependent on each other. By physically forming complexes, they are able to execute coordinated histone acetylation and deacetylation rapidly in the same region of chromatin.[153]

An important example is CBP/p300, which has been shown to acetylate various histone lysine residues, including H2A (K5), H2B (K12, K15), H3 (K14, K18, K27, K36, K56), and H4 (K5, K8, K12),[154,155] and to act as a limiting transcriptional coactivator utilized by many DNA-binding proteins, including p53, E2F, and NF-κB, to facilitate transcriptional activation. SIRT1 regulates the activity of p300 by two different mechanisms. SIRT1 deacetylates p300 at two lysine residues (K1020 and K1024), leading to the SUMO modification of these two residues and transcriptional repression of p300.[156] SIRT1 also destabilizes p300 by deacetylation and promoting ubiquitination in dystrophic heart.[157] Since SIRT1 and p300 regulate cellular function reciprocally by deacetylating and acetylating proteins, respectively, SIRT1-mediated negative regulation of p300 might promote its effect on chromatin silencing and transcriptional repression.

The MYST acetyltransferase family members, hMOF[158,159] and TIP60,[159,160] are also SIRT1 deacetylase substrates. SIRT1 binds and deacetylates the enzymatic domains of hMOF and TIP60, leading to the repression of their HAT activity and ubiquitination-dependent degradation of these proteins.[159,161] Notably, hMOF is necessary for the acetylation of H4K16. Since H4K16 is the primary histone target of SIRT1 and exerts an important role in higher order chromatin organization, SIRT1 might control the process not only by deacetylating H4K16 but also by inhibiting hMOF. Interestingly, under DNA damage conditions, the binding between SIRT1 and hMOF/TIP60 decreases, activating hMOF/TIP60 for DNA double-strand break repair.[159]

3.1.2.2. SIRT1 and Histone Methylation.

Deacetylation of nucleosome histones by SIRT1 is able to alter methylation of histones. SIRT1 recruits and activates histone methyltransferase (HMT) at the target sites and therefore regulates both histone acetylation and methylation. As described above, RNAi-mediated SIRT1 depletion renders hypomethylation of H3K9me3, which is a hallmark of heterochromatin in eukaryotes. Later, it was elucidated that SIRT1 promotes H3K9me3 during FH formation by activating the main H3K9 methyltransferase, SUV39H1 (suppressor of variegation 3−9 homologue 1).[162] SIRT1 directly binds to and recruits SUV39H1 to genomic loci and activates its HMT activity by deacetylating the Lys266 in its catalytic domain.[163] SIRT1, SUV39H1, and Nucleomethylin exist in a complex and eNoSC (energy-dependent nucleolar-silencing complex) in the rDNA loci.[164,165] Energy depletion-induced increase in NAD/NADH ratio activates SIRT1, resulting in deacetylation of H3K9 and SUV39H1-mediated H3K9me2, establishment of silent chromatin in the loci, and subsequent repression of rRNA transcription.[164] SIRT1 is also linked to SUV39H1-dependent

constitutive heterochromatin formation and genome protection in response to oxidative stress. SIRT1 competes with the E3 ubiquitin ligase of SUV39H1, MDM2, for binding to SUV39H1, thereby inhibiting its degradation through ubiquitination.[166]

In addition to SUV39H1, EZH2, another polycomb group HMT that targets H3K27 and H1K26 for methylation, was also linked to the effect of SIRT1 on histone methylation. SIRT1 and EZH2 are found to coexist in the PRC4 complex (polycomb repressive complex 4), which is detectable only in undifferentiated embryonic stem (ES) cells and some EZH2-overexpressing cancer cells. Although EZH2-containing PRC complexes usually preferentially methylate H3K27, with the presence of SIRT1, PRC4 specifically targets H1K26 for methylation. This finding suggests that the deacetylation of H1K26ac by SIRT1 may coordinate the methylation of H1K26 by EZH2.[167]

SIRT1 also acts in concert with histone demethylases to control gene expression. It forms corepressor complex with the histone H3K4 demethylase LSD1/KDM1A (lysine-specific demethylase 1A) and plays conserved and concerted roles in deacetylation of H4K16 and demethylation of H3K4 to repress NOTCH target genes during development.[168] Lastly, SIRT1 arrival also promotes H4K20me1 and loss of H3K79me2, suggesting the coordination between SIRT1 and other methyltransferase and demethylase during heterochromatin formation. Yet the mechanism remains to be elucidated.[128]

3.1.2.3. SIRT1 and DNA Methylation.

Multiple reports suggested that SIRT1 is associated with DNA methylation and colocalizes with the DNA methylation machinery in hypermethylated chromatin regions. Pruitt et al. showed that SIRT1 localizes to promoters of various silenced tumor suppressor genes with 5′ CpG island hypermethylation but not to the same promoters that are not hypermethylated. Inhibition of SIRT1 in breast and colon cancer cells resulted in reactivation of the silenced tumor suppressor genes.[169] Studies by Espada et al. indicated that DNMT1 (DNA-methyltransferase 1) interacts with SIRT1 and serves as an epigenetic caretaker to maintain the nucleolar structure through recruitment of SIRT1.[170]

Using an exogenous reporter construct containing *E-cadherin* promoter, O'Hagan et al. illustrated that SIRT1, EZH2, DNMT1, and DNMT3b were recruited to DNA double-strand breaks and that SIRT1 is required for transient recruitment of DNMT3b and subsequent aberrant DNA methylation in the exogenous promoter CpG island.[171] In line with this, a follow-up genome-wide study revealed that a large silencing complex containing DNMT1, DNMT3b, SIRT1, and other PRC4 members are relocalized to CpG islands of gene promoters upon oxidative damage. Such translocation causes aberrant DNA methylation and transcriptional silencing, indicating the involvement of SIRT1-dependent DNA methylation in DNA damage-induced epigenetic silencing in tumors.[172] Stable interaction between DNMT3b and SIRT1 was also detected in the condensed chromatin, suggesting that SIRT1 promotes recruitment of DNMT3b and the onset of DNA methylation.[173]

Furthermore, SIRT1 deacetylates DNMT1 and alters its activities. Interestingly, SIRT1-induced deacetylation of DNMT1 shows domain-specific consequences. Deacetylation of the C terminus catalytic domain increases its methyltransferase activity, whereas deacetylation of the GK linker impairs the transcription repression activity independent of its methyltransferase activity.[174] These seemingly contradictory effects

might be due to the fact that different domains regulate the functions of DNMT1 independently and that the deacetylation of distinct domains of DNMT1 may occur in a context-dependent manner. Although details of the mechanism remain to be further studied, these observations provide novel insight into the functional diversity of SIRT1 in DNA methylation and epigenetic gene silencing.

Additional evidence supporting the coordinating role of SIRT1 in heterochromatin formation came from a study by Zhou et al. They showed that SIRT1 deacetylates TIP5 (transcription termination factor I-interacting protein 5), the largest subunit of the SNF2h-containing chromatin remodeling complex NoRC (nucleolar remodeling complex). NoRC silences rRNA gene expression by establishing histone H4K16 deacetylation, H3K9 dimethylation, and DNA methylation at the rRNA promoter. Deacetylation of TIP5 by SIRT1 increases the binding of NoRC to promoter-associated RNA (pRNA), which is complementary to the rRNA promoter and essential for NoRC function. Therefore, SIRT1 promotes the NoRC-mediated establishment heterochromatin histone marks, DNA methylation, and rRNA silencing, further highlighting its function in heterochromatin formation.[175]

3.1.3. SIRT1 and Constitutive Heterochromatin.

Growing evidence suggests that SIRT1 is involved in the formation of not only FH but also constitutive heterochromatin (CH), including the pericentromeric and telomeric heterochromatin. SIRT1 was found to localize to and contribute to the silencing of pericentromeric major satellite repeats in mouse ES cells.[81] $Sirt1^{-/-}$ MEFs showed a complete loss of H3K9me3 and localization of heterochromatin protein HP1 at the pericentromeric CH loci in over 50% of the cells tested.[163] A similar phenomenon was observed in $Sirt1^{-/-}$ brain compared with the $Sirt1^{+/+}$ brain, suggesting the involvement of SIRT1 in SUV39H1-dependent CH formation.[176] Supporting the direct effect of SIRT1 on CH, expression of exogenous wild type but not catalytic mutant of SIRT1 in $Sirt1^{-/-}$ MEFs rescued the loss of H3K9me3 in CH regions.[163] Later, it was elucidated that SIRT1 regulates CH by stabilizing SUV39H1 in CH regions as discussed above.[166]

The role of SIRT1 in telomere maintenance is still under debate. On one hand, it was observed that mouse cells deficient in SIRT1 exhibit spontaneous telomeric abnormalities associated with impaired cell growth.[177] SIRT1 interacts with telomeric repeats in vivo and positively regulates telomere length by decreasing the rate of telomere erosion.[178] In line with these findings, SIRT1 silencing causes telomere dysfunction and repressed proliferation of HCC (hepatocellular carcinoma cells) associated with decreased expression of TERT (telomerase reverse transcriptase) and PTOP (POT1- and TIN2-organizing protein).[179] However, on the other hand, there is evidence showing that SIRT1 inhibits the expression of TERT and suppresses the growth of certain cell lineages, suggesting the role of SIRT1 as a barrier to retard the growth of certain nutrient-sensitive cells.[180]

3.1.4. SIRT1 Deacetylates and Regulates Transcription Factors.

SIRT1 can also interact with and deacetylate numerous transcription factors, thereby modulating their transactivation activity to activate or suppress genes. By modulating these transcription factor-dependent expression programs, SIRT1 is involved in the regulation of stress response, metabolism, cell differentiation, cell survival, and circadian clock. These transcription factors include p53, PGC-1α (peroxisome proliferator-activated receptor gamma coac-

tivator 1-α), FOXOs (forkhead box O transcription factors), HIF-1α and HIF-2α (hypoxia-inducible factor 1α and 2α), NF-κB, and MYC. Since numerous transcription factors have been reported as SIRT1 substrates, we list them in Table 1 and only discuss some of them in the following section.

3.1.4.1. SIRT1 Regulates Transcription Factors Controlling Metabolism. SIRT1 is known to be involved in various metabolic processes in response to nutrient availability. During fasting, SIRT1 regulates gluconeogenesis and fatty acid oxidation by deacetylating several transcription factors, including CRTC2 (CREB-regulated transcription coactivator 2), PGC-1α, FOXO1, and SREBP1 (sterol regulatory element-binding protein 1). This is one of the best established models about how SIRT1 modulates stress response through deacetylating specific transcription factors. During the early stage of fasting, the fasting hormone glucagon activates CRTC2 by facilitating its nuclear localization and acetylation by p300, which then stimulates the gluconeogenic gene expression. During late fasting, SIRT1 is activated to deacetylate CRTC2, which leads to the ubiquitination and degradation of CRTC2.[181] At the same time, SIRT1 deacetylates and activates FOXO1[181] and its coactivator PGC-1α,[182] resulting in the activation of genes involved in the late stage of gluconeogenesis. However, STAT3 (signal transducer and activator of transcription 3) antagonizes the activity of FOXO1/PGC-1α and inhibits the gluconeogenic gene expression. SIRT1 suppresses the inhibitory effect of STAT3 by deacetylation, thereby maximizing the activation of gluconeogenesis.[183] The deacetylation of PGC-1α also coactivates PPARα to enhance the expression of mitochondrial fatty acid oxidation genes.[184] Moreover, SREBP1, an important transcription factor that controls lipid and sterol homeostasis, could also be deacetylated by SIRT1 during fasting.[185] Deacetylation of SREBP1 represses its protein stability and transactivation of target genes, thereby blocking synthesis of lipid and cholesterol.

3.1.4.2. SIRT1 Regulates Transcription Factors Involved in Stress Response. A large number of studies have demonstrated that SIRT1 mediates different types of stress response, such as oxidative stress, DNA damage, inflammation, hypoxic stress, and so on. For example, SIRT1 forms a complex with and deacetylates FOXO3a[186] in response to oxidative stress, which has a dual effect on the activity of FOXO3a. On one hand, SIRT1 activates FOXO3a to induce cell cycle arrest and resistance to oxidative stress. On the other hand, SIRT1 inhibits the ability of FOXO3a to induce cell death. Thus, SIRT1 protects cells from stress-induced cell death by tipping the balance toward stress resistance and survival.[187]

The p53 protein is the first reported nonhistone substrate of SIRT1. SIRT1 deacetylates p53 at its C-terminal Lys382 residue and alleviates its transactivation activity. SIRT1 therefore is able to protect cells from p53-induced apoptosis[188] and senescence.[189] Notably, p53 also regulates SIRT1 in a positive feedback loop. The binding of p53 to the *Sirt1* promoter inhibits SIRT1 expression under normal nutrient conditions.[190] Also, p53 stimulates the expression of miR-34a, which represses SIRT1 and therefore SIRT1-mediated deacetylation and inhibition of p53.[191] Hence, the SIRT1-p53 axis is implicated in the development of various cancers.

SIRT1 could also attenuate inflammation by regulating NF-κB. SIRT1 deacetylates the RelA/p65 subunit of NF-κB at Lys310 and represses its transcription activity, thereby sensitizing cells to TNF-α-induced apoptosis.[192] It has also been reported that SIRT1 deacetylates and destabilizes FOXP3

Table 2. SIRT2 Deacetylation Substrates

substrate	full name	modified lysine residues	function of SIRT2-catalyzed deacetylation
histones			
H4	histone H4	K16	regulate chromatin condensation during metaphase, regulate H4K20 methylation, promote cell cycle progression and genome stability; suppress transcription of certain genes[300−302]
H3	histone H3	K18	*L. monocytogenes* InlB triggers SIRT2 nuclear localization to suppress gene transcription;[303] involved in DNA replication and DNA damage repair[150,304]
		K56	unclear, may be involved in DNA damage repair
transcription factors			
P300		many	promote binding of p300 to preinitiation complex[305]
FOXO3	forkhead box protein O3	unknown	increase FOXO3 DNA binding and target gene transcription;[148,306] increase FOXO3 ubiquitinylation and degradation[234]
FOXO1	forkhead box protein O1	unknown	promote FOXO1 interaction with PPARγ and repress PPARγ target genes;[307,308] inhibit FOXO1 interaction with ATG7 and autophagic cell death[309]
HIF-1α	hypoxia-inducible factor 1α	K709	promote hydroxylation and degradation of HIF-1α[310]
NF-κB	nuclear factor κ B	K310	suppress NF-κB-dependent gene expression[311]
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α	unknown	decrease expression of β-oxidation and mitochondrial genes[312]
cell cycle related			
BubR1	mitotic checkpoint serine/threonine-protein kinase BUB1 β	K688	stabilize BubR1, improve cardiac function, and extend lifespan in vivo[313]
CDK9	cyclin-dependent kinase 9	K48	increase CDK9 kinase activity and decrease sensitivity to hydroxyurea-induced replication stress response[314]
CDH1/CDC20	CDH1/cell-division cycle protein 20	K69 and K159 (CDH1), K66 (CDC20)	activate the E3 ubiquitin ligase activity, leading to decreased Aurora A level[315]
metabolic enzymes			
LDH-A	lactate dehydrogenase A	K5	activates LDH-A[316]
PEPCK	phosphoenolpyruvate carboxykinase	K70, K71	inhibit the ubiquitinylation and degradation of PEPCK[317]
ACLY	ATP-citrate lyase	K540, K546, K554	promote ATP-citrate lyase degradation[318]
G6PD	glucose-6-phosphate dehydrogenase	K403	promote the formation of active G6PD dimer and increase NADPH production[319]
PGAM	phosphoglycerate mutase	K100	activate PGAM activity[320]
cell signaling related			
PRLR	prolactin receptor	many	facilitate prolactin receptor dimerization and activation of STAT5[321]
K-Ras	Kirsten rat sarcoma viral oncogene homologue	K104	promote K-Ras activity[322]
PAR-3	partitioning defective 3 homologue	K831, K848, K881, K1327	decrease the activity of aPKC and regulate myelin formation[323]
TIAM1	T-cell lymphoma invasion and metastasis 1	K1420	promote activation of DVL/TIAM1/Rac axis and cell migration in cancer cells[295]
structural proteins			
keratin 8	keratin 8	K207	affect its phosphorylation and filament organization[324]
α-tubulin	α-tubulin	K40	destabilize microtubule[325]

(forkhead box protein P3), which is essential for the functionality of regulatory T cells (Treg).[193−196]

SIRT1 binds to and deacetylates HIF-1α at Lys674 and inhibits the transactivation of HIF-1α to control the glycolysis in response to hypoxic stress. By doing so, SIRT1 negatively regulates the growth and angiogenesis of fibrosarcoma tumors in vivo.[197] SIRT1 also deacetylates HIF-2α, which is closely related to HIF-1α in structure but differs from HIF-1α in the transcriptional targets. During hypoxia, SIRT1 deacetylates and activates HIF-2α signaling and the corresponding hypoxic stress response.[198] Notably, HIF-1α and HIF-2α are regulated by SIRT1 oppositely. Given that during hypoxia, the expression and activity of SIRT1 gradually decrease as NAD level decreases, it has been proposed that the deactivation of SIRT1 may trigger a switch from HIF-2α to HIF-1α, thereby coordinating hypoxic stress response and hypoxic metabolism.[197]

3.1.4.3. SIRT1 Regulates Tumor-Promoting Transcription Factors. SIRT1 could also promote cell proliferation by forming a positive feedback loop with the oncoprotein MYC. C-MYC increases SIRT1 expression, which in turn deacetylates and enhances its transcriptional activity.[199−202] Constitutive activation of this SIRT1-C-MYC positive feedback loop promotes C-MYC-induced cell proliferation by suppressing apoptosis and senescence.[199,202] SIRT1 also promotes N-MYC oncogenesis in neuroblastoma through a positive feedback loop involving MKP3 (mitogen-activated protein kinase phosphatase 3) and ERK (extracellular-signal-regulated kinases). However, unlike C-MYC, N-MYC is not a deacetylase substrate of SIRT1. Instead, SIRT1 and N-MYC form a transcriptional repressor complex at gene promoter of MKP3, leading to repression of MKP3 expression, ERK protein phosphorylation, N-MYC phosphorylation, and stabilization.[200]

3.1.4.4. SIRT1 Regulates Transcription Factors in Circadian Clock. The core players of the circadian clock machinery are

the transcription factors CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle ARNT-like 1), which heterodimerize, bind, and activate the transcription of clock controlled genes (CCGs), such as *Per* (*Period*) and *Cry* (*Cryptochrome*). When PER and CRY proteins accumulate to a certain level, they form a complex with CLOCK-BMAL1 heterodimer and inhibit their own transcription. In peripheral tissues such as liver, SIRT1 binds to CLOCK-BMAL1 rhythmically and promotes the deacetylation-dependent degradation of PER2, thereby derepressing the transactivation of CLOCK-BMAL1.[203] CLOCK has HAT activity and is able to acetylate histone H3 and its partner BMAL1. Acetylation of BMAL1 facilitates CRY binding and thus CRY-mediated transcriptional repression. SIRT1 is recruited by CLOCK/ BMAL1 and deacetylates BMAL1 and histone H3K9/H3K14 at the promoters of CCGs in a timely manner. Deacetylation of BMAL1 releases it from CRY-mediated transcriptional repression, whereas deacetyaltion of histone H3 by SIRT1 negatively regulates the CCGs transcription. Interestingly, it has been shown that the oscillations in the acetylation pattern of BMAL1 and H3 differ in the timing, which is in line with the dual role of CLOCK-induced acetylation of BMAL1 (repression) and H3 (activation).[204]

Recently, SIRT1 is linked to central circadian control in the SCN (suprachiasmatic nucleus). In the brain, SIRT1 activates the transcription of CCGs, including *Bmal1* and *Per2*, by cooperatively binding with and deacetylating PGC1-α at the promoters of CCGs. Notably, SIRT1 modulates the central circadian clock by a mechanism that becomes less efficient in aged animals. It has been reported that NAMPT (nicotinamide phosphoribosyltransferase), a key enzyme that controls the NAD salvage pathway, is also a CCG that is controlled by the circadian machinery containing SIRT1.[205] Thus, when the NAD levels decrease during aging,[206] SIRT1 becomes less active and the circadian control decays. Therefore, it is also proposed that SIRT1 acts as a rheostat to transduce signals generated from cellular metabolism to the circadian clock control.[204]

3.2. SIRT2 in Epigenetic Regulation

SIRT2 is connected with multiple cellular processes, including mitosis, cell cycle, cell death, metabolism, and aging. SIRT2 was initially reported to be mainly in the cytoplasm, colocalizes with microtubules, and deacetylates α-tubulin at Lys40.[298] It was recently reported to regulate many other cytosolic proteins, including LDH-A (lactate dehydrogenase A), PEPCK1 (phosphoenolpyruvate carboxykinase 1), ACLY (ATP-citrate lyase), and G6PD (glucose-6-phosphate dehydrogenase) (see Table 2). It was later found that SIRT2 can translocate to the nucleus and has important epigenetic roles. A nuclear specific isoform of SIRT2 was also reported, but it has no deacetylase activity.[299]

3.2.1. SIRT2 Deacetylates Histones and Regulates Cell Cycle, DNA Repair, and Transcription.
SIRT2 can deacetylate H4K16 and regulate chromatin structure during cell cycle and DNA damage. SIRT2 level is regulated by cell cycle. During mitosis, SIRT2 expression level increases and translocates to the nucleus.[326] Deletion of SIRT2 leads to increased H4K16 acetylation in mitosis and affects the cell cycle.[327] Interestingly, the hyperacetylation in *Sirt2*−/− mice also leads to loss of H4K20me1, a histone mark that is established in G2/M phase by the methyltransferase PR-SET7 and is important for cell cycle progression and DNA repair.[301]

H4K16 acetylation directly inhibits the methylation of H4K20 by PR-SET7.[301] In addition, SIRT2 can also bind to PR-SET7 and deacetylate it at Lys90. SIRT2 binding increases PR-SET7 activity, and deacetylation promotes its recruitment to the chromatin.[301] The decrease in H4K20me1 is thought to be responsible for the defect in pericentromeric heterochromatin and in DNA repair. Correspondingly, *Sirt2*−/− mice suffer from greater DNA damage and genome instability.[301] Increased tumorigenesis in *Sirt2*−/− mice was observed in a DNA-damage-induced skin tumor model. However, in contrast to the previous report,[315] no increased spontaneous tumorigenesis was observed in the knockout mice up to 1 year of age.[301]

Unlike SIRT1, the gene-silencing function of SIRT2 is less well known. However, a few examples do exist. SIRT2 is known to suppress the transcription of keratin 15/19,[328] ARRDC3 (arrestin domain-containing 3),[329] and NEDD4 (neural precursor cell expressed developmentally downregulated protein 4).[302] The H4K16 deacetylation activity likely underlies the repression function of SIRT2 on these genes. However, it is not clear how SIRT2 is specifically recruited to these genes.

Interestingly, SIRT2 is reported to function as an H3K18 deacetylase in the context of *Listeria monocytogenes* infection.[303] In a series of elegant studies, Eskandarian and co-workers demonstrated that the InlB protein of *L. monocytogenes* activates the PI3K/AKT signaling pathway, leading to the nuclear translocation of SIRT2. The nuclear translocation may be mediated by a posttranslational modification of SIRT2. However, the detail is not clear. Most strikingly, SIRT2 in this case does not deacetylate H4K16, but instead it deacetylates H3K18. The deacetylation of H3K18 is thought to lead to the suppression of a set of genes during *L. monocytogenesis* infection. Treatment with a SIRT2-specific inhibitor, AGK2, inhibited the deacetylation of H3K18 and transcriptional suppression. It is proposed that *L. monocytogenesis* uses this mechanism to manipulate the host cell to maximize their survival and proliferation. Indeed, inhibition or knockdown of SIRT2 significantly impaired *L. monocytogenesis* infection.[303] It is tempting to speculate that SIRT2-catalyzed H3K18 deacetylation and transcriptional regulation also occur in mammalian cells in the absence of bacterial infection. Eskandarian and co-workers thought this is unlikely as SIRT2 inhibition by AGK2 did not change the gene expression profile in the absence of *L. monocytogenes* infection. Future studies will be required to further validate this.

SIRT2 has also been reported to deacetylate H3K56.[150,304] H3K56 acetylation level increases during S phase and in DNA damage foci, colocalizes with double-strand break markers, such as γ-H2AX, pATM, CHK2, and p53.[150,304] In yeast, histone H3K56 acetylation has been shown to signal the completion of DNA repair and facilitate the reassembly of nucleosomes at the repaired sites.[92] If the same applies in mammalian cells, SIRT2 may function to remove the H3K56 acetylation after DNA repair.

3.2.2. SIRT2 Deacetylates Transcription Factors and Regulates Transcription.
SIRT2 can also deacetylate several transcription factors or coactivators, thereby exerting effects on transcription of specific genes. In this case, the effects on transcription can be either positive or negative. SIRT2 was shown to deacetylate p300 and promote binding of p300 to the preinitiation complex.[305] FOXO1 and FOXO3 are both known to be deacetylation targets of SIRT2. FOXO1 deacetylation by SIRT2 promotes FOXO1 interaction with PPARγ and represses PPARγ target genes.[307,308] Deacetylation by SIRT2

also inhibits FOXO1 interaction with ATG7 and autophagic cell death.[309] For FOXO3, SIRT2-catalyzed deacetylation increases DNA binding and target gene transcription in one report[306] and increases FOXO3 ubiquitination and degradation in another.[148] HIF-1α can be deacetylated by SIRT2, which promotes its hydroxylation and degradation.[310] NF-κB and PGC-1α are also deacetylation targets of SIRT2.[311,312] For both proteins, deacetylation suppresses their target gene expression.

The regulation on transcription factors by SIRT2 can also be indirect. For example, SIRT2 has recently been shown to stabilize the C-MYC and N-MYC oncogenic transcription factors in neuroblastoma and pancreatic cancer cells.[302] This is achieved by suppressing the transcription of the ubiquitin ligase NEDD4 via histone H4K16 deacetylation.[302]

3.3. SIRT6 in Epigenetic Regulation

Among the seven mammalian sirtuins, SIRT6 is the second sirtuin, next to SIRT1, whose deletion in mice causes very severe phenotypes, including severe metabolic defects, genome instability, and premature aging.[330] Further studies revealed that SIRT6 regulates many important pathways via epigenetic mechanisms, mainly histone deacetylation. SIRT6 itself is regulated by several different mechanism, including p53, AP-1 (activator protein 1), and SIRT1-mediated transcription control,[331−333] as well as phosphorylation by AKT1, MDM2-mediated ubiquitination, and UCP10 (UBX domain-containing protein 10)-mediated deubiquitintion at posttranslational level.[334,335]

3.3.1. SIRT6 in Gene Silencing.
SIRT6 has a very weak deacetylase activity toward peptide substrates in vitro. By screening a series of histone peptides, Chua and others identified that it can specifically deacetylate H3K9[41] and H3K56,[42,43] although the activity is still fairly weak compared to SIRT1. More recently, Cohen and co-workers found that SIRT6 deacetylates histones when they are packaged as nucleosomes but not as free histones,[48] suggesting that the deacetylase activity of SIRT6 is nucleosome dependent. Additionally, Denu and co-workers' study revealed that SIRT6's ability to remove acetyl group from histone peptides could also be activated by the existence of free fatty acids, such as myristic, oleic, and linoleic acids.[49]

At present, most of the gene-silencing effects of SIRT6 have been explained by its H3K9 deacetylase activity. SIRT6 is recruited to HIF-1α target gene promoters and suppresses the transcription by deacetylating H3K9.[332,336] Similarly, SIRT6 is shown to be recruited to MYC,[46] NF-κB,[47] C-JUN,[337,338] and FOXO3[339,340] target gene promoters and suppress the transcription of these genes. The recruitment of SIRT6 by specific transcription factors to help suppress gene transcription via H3 deacetylation have been used to explain many phenotypes associated with SIRT6 deficiency, including tumor development,[46] cardiac hypertrophy,[338] growth retardation,[341] and lipid metabolism/liver inflammation.[339,340] This mechanism has similarly been used to explain the phenotype of SIRT6 overexpressing transgenic mice, including the decreased low-density lipoprotein cholesterol levels[340,342] and increased lifespan in male mice.[343] The mechanism underlying the lifespan extension effect caused by SIRT6 overexpression, however, is not completely understood. The major gene expression change induced by SIRT6 overexpression that contributes to the lifespan extension is the increased IGFBP1 (insulin-like growth factor binding protein 1) level.[343] How SIRT6 increases IGFBP1 expression cannot be directly

explained using the simple model described above. It is possible that SIRT6 suppresses the expression of another factor that can suppress IGFBP1 expression.

A genome-wide CHIP-Chip analysis showed that SIRT6 binds to the promoters of about 2000 genes in mouse fibroblasts.[344] SIRT6 chromatin localization has significant overlap with that of NF-κB. Furthermore, the localization is very dynamic. TNFα stimulation causes the release of SIRT6 from a large portion of the 1900 genes and the relocalization to an even larger number (∼4300) of different genes.[344] Despite the fact that SIRT6 deletion changes the expression of many NF-κB target genes, there is a report that overexpression of SIRT6 in mice does not change the expression of NF-κB target genes.[345] This was also observed in the studies by Cohen and co-workers on the lifespan-extending role of SIRT6 in male mice.[343] A model that could explain these observations is that at resting state SIRT6 binds to promoters of NF-κB target genes to suppress the basal expression. There is perhaps enough SIRT6 to suppress the basal expression of target genes, and thus, overexpression of SIRT6 does not further increase the suppression. When there is an external stimulation (e.g., TNFα), NF-κB will be turned on more strongly, which leads to dissociation of SIRT6, releasing the suppression.

SIRT6 is demonstrated to be required for the telomere position effects in mammalian cells,[346] similar to the role of yeast SIR2 discussed above. Knockdown of *Sirt6* increased the expression of both an integrated luciferase reporter gene and an endogenous telomere-proximal gene. The silencing effect requires the deacetylase activity of SIRT6 and is associated with decreased H3K9 acetylation and increased H3K9 methylation.[346]

SIRT6 has also been reported to deacetylate transcription factors or coactivators. GCN5 was shown to be a deacetylation target of SIRT6.[347] Deacetylation of GCN5 increases its acetyltransferase activity, leading to increased PGC-1α acetylation and decreased gluconeogenesis gene expression.[347] This is in contrast to the role of SIRT1 in PGC-1α regulation, which directly deacetylates PGC-1α and activates gluconeogenesis gene expression. SIRT6 has recently been reported to deacetylate transcription factor FOXO1 and regulates the expression of gluconeogenesis genes.[331] The tumor suppressor p53 upregulates SIRT6 expression, which promotes the deacetylation and nuclear exclusion of FOXO1, leading to downregulation of gluconeogenesis genes.[331] The deacetylation of other transcription factors by SIRT6, such as HIF-1α, was suspected but not detected.[102] However, the deacetylation of both GCN5 and FOXO1 by SIRT6 was only demonstrated in vivo but not in vitro. Thus, it remains possible that the deacetylation of FOXO1 and PGC-1α by SIRT6 is indirect, and further validation will be helpful.

SIRT6 has also been indicated to control the circadian clock in mice liver.[348] The circadian expression of many liver genes is affected by SIRT6, which is distinct and shows only partial overlap with circadian gene expression controlled by SIRT1. This is an interesting observation, but mechanistically this seems to be different from the silencing role of SIRT6 described above for other transcription factors, such and NF-κB and HIF-1α. Existing data suggest that SIRT6 can bind to CLOCK and BMAL1. SIRT6 also decreases the binding of BMAL1 and SREBP1 at target gene promoters. The role of SIRT6 in BMAL1 and SREBP1 regulation seems to resemble that in FOXO1 regulation but without deacetylating CLOCK/BMAL1 or SREBP1. This example also illustrates that the role

of SIRT6 in transcriptional regulation is still not well understood.

3.3.2. SIRT6 in Genome Stability and DNA Repair.

SIRT6 plays several distinct roles in DNA repair and genome stability. *Sirt6* mice display genome instability.[330] SIRT6-deficient cells have increased sensitivity to ionizing radiation, monofunctional alkylating reagents, and hydrogen peroxide but normal sensitivity to UV-induced DNA damage or endonuclease induced double-strand break, which suggest that SIRT6 is important for base excision repair (BER).[330] PARP1 is another protein that is involved in BER; however, PAR foci formation in *Sirt6* knockout cells were not affected, suggesting that SIRT6 may be acting downstream of PARP1.[330] *Sirt6* deletion leads to end-to-end chromosomal fusion and abnormal telomere structures that resemble defects observed in Werner syndrome.[41] Later, SIRT6 was also found to be important for DNA double-strand break repair.[349,350] The molecular role of SIRT6 in DNA repair is not entirely clear and likely multifacet. The telomere fusion phenotype has been explained by a model that SIRT6-catalyzed H3 K9 deacetylation forms a special chromatin state at telomeres, which is required to recruit WRN, the factor that is mutated in Werner syndrome. During DNA double-strand repair, it has been shown that SIRT6 deacetylates H3K9 at DNA damage site and helps to recruit DNA-PK (DNA-dependent protein kinase).[349] SIRT6 also promotes DNA end resection by deacetylating CtIP (CtBP-interacting protein), a protein involved in DNA end resection.[350] Mao et al. also reported that SIRT6 can ADP-ribosylate PARP1 to increase PARP1 activity and promote DNA repair.[376] Recently, SIRT6 is reported to be one of the earliest enzymes recruited to DNA double-strand breaks and promotes the recruitment of a chromatin remodeling protein, SNF2H, and deacetylates H3K56. The involvement of chromatin remodeler and histone deacetylation suggests that similar to DNA repair in yeast discussed earlier, dynamic chromatin structure changes are required for DNA repair.[377]

3.4. Role of SIRT7 in Epigenetic Regulation

Similar to SIRT6, SIRT7 is also a class IV sirtuin that is mainly localized in the nucleus.[351] It is specifically enriched in the nucleolus.[351] The nucleolus localization is dependent on active RNA Pol I transcription.[352] In addition, significant cytoplasmic localization of SIRT7 has also been reported.[353] Many interacting proteins have been identified, many of which are associated with ribosome biogenesis.[354−356]

SIRT7 has low deacetylase activity,[351] and because of this, its first deacetylation substrate was only identified in 2012. By screening a set of acetyl lysine peptides, Chua and co-workers discovered that SIRT7 can specifically deacetylate H3K18.[357] This elegant study demonstrated that SIRT7 is recruited to the promoters of a set of genes, many of which are controlled by a transcription factor called ELK4. SIRT7 is recruited by ELK4 to the promoters of target genes, leading to H3K18 deacetylation and suppression of transcription.[357] Recently, MYC was identified as another transcription factor that also recruits SIRT7 to target gene promoters (mainly genes encoding ribosome proteins) to suppress transcription via H3K18 deacetylation.[358] Interestingly, it was shown that SIRT7 attenuate ER (endoplasmic reticulum) stress and prevents fatty liver formation in mice by suppressing MYC target genes. The model proposed was that under ER stress, SIRT7 suppresses translation by suppressing ribosomal protein synthesis, thus relieving the protein folding pressure in the ER. However, this is controversial as another report showed that SIRT7 promotes fatty liver formation under a high-fat diet.[359] This latter report by Yoshizawa et al. showed that SIRT7 can bind to an E3 ubiquitin ligase complex DCAF1/DDB1/CUL4B and inhibits its activity in promoting TR4 (testicular receptor 4) degradation. It has been reported that TR4 deficiency protects mice from high-fat diet-induced hepatic steatosis. Thus, without SIRT7, TR4 level decreases, which leads to decreased expression of genes that promote lipid deposition, such as CD36 and CIDEA, and protects mice from high-fat diet-induced fatty liver. The SIRT7 effect on TR4 can at least partly explain the phenotype of *Sirt7* knockout.[359]

SIRT7 is unique in the sense that it can regulate both RNA Pol II transcription by working with different transcription factors as described above and RNA Pol I transcription. One of the earliest effects of SIRT7 reported was its ability to positively regulate RNA Pol I transcription.[352] The mechanism underlying the effect was found to be SIRT7-mediated deacetylation of the PAF53 subunit of RNA Pol I.[360] Deacetylation of PAF53 leads to increased association of RNA Pol I with rDNA and increased rRNA transcription.[360]

4. SUMMARY AND OUTLOOK

The studies on sirtuins discussed above provided a number of important lessons in chromatin biology and epigenetics. These studies also revealed many unresolved fundamental questions. Here we discuss a few of the lessons and questions that may be useful for guiding future studies.

4.1. Value of Model Organisms

Sirtuins have attracted many researchers, and the number of publications on sirtuins have increased dramatically in the past decade. Looking back, it is stunning that this prosperous research area was initiated from some basic genetic studies in a simple model organism, the budding yeast. Obviously, there are important differences between yeast and mammalian sirtuins. The biological function of mammalian sirtuins is more complex and quite different from that of yeast. However, the basic enzymatic function is conserved, and many general trends are similar. Thus, it is important to appreciate the value of model organisms.

4.2. Connection between Sirtuins, Metabolism, and Epigenetics

The connection between sirtuins, metabolism, and epigenetics is manifested in several aspects. First, most of the protein acyl lysine modifications use acyl-CoA molecules as the acyl donors, which are common metabolites in cells. Thus, metabolism can affect these PTMs, including the epigenetic modifications on histones, by changing the concentrations of the acyl-CoA molecules. The absolute requirement of NAD as a cosubstrate for sirtuin-catalyzed deacylation suggests that sirtuins can act as NAD sensors that transduce metabolism signals to epigenetic regulations of gene expression. It has been reported that NAD levels increase in muscle and white adipose tissue upon caloric restriction, thereby activating sirtuins.[361] Indeed, it was found in many studies that SIRT1 activity is low in conditions of glucose excess and high in conditions of nutrient limitation.[362] As discussed above, it has been well established that SIRT1 regulates metabolic responses to changes in nutritional availability in multiple tissues. However, it still remains elusive how other mammalian sirtuins respond to changes in metabolism and how the responses affect epigenetic marks in cells. The observation that SIRT6 can be activated by free fatty

Table 3. Comparison of the Deacetylation and Defatty-Acylation Activities of SIRT6

acyl peptide	k_{cat} (S^{1-})	K_m (μM)	k_{cat}/K_m (S^{-1} M^{-1})
H3K9 acetyl	0.0039 ± 0.0006[365]	810 ± 160[365] or $\sim$450[49]	4.8[365] or 6.4 ± 2[49]
H3K9 acetyl with myristic acid	$\sim$0.002[49]	9 ± 1[49]	230 ± 30
H3K9 myristoyl	0.0049 ± 0.0004[365]	3.4 ± 0.9[365]	1.4×10^3[365]
H3K9 palmitoyl	0.0027 ± 0.0002[365]	0.9 ± 0.4[365]	3.0×10^3[365]

acids suggests that sirtuins may be regulated by metabolites other than NAD. It remains to be elucidated whether other metabolites regulate sirtuins and how the regulation contributes to the epigenetic roles of sirtuins.

4.3. Recruitment of Mammalian Sirtuins to Different Regions of Chromatin or DNA Damage Sites

For yeast SIR2, the recruitment to the silencing loci is relatively well understood. Such understanding for mammalian sirtuins is only partial. For example, how SIRT1 is recruited to certain gene promoters or DNA damage sites is not completely known. The recruitment of SIRT6 by different transcription factors is relatively clearer. However, given that SIRT6 interacts with many different transcription factors, what determines the binding specificity is not clear. The recruitment of SIRT2 to chromatin is even more mysterious as no transcription factor is known to bind to SIRT2. Furthermore, because SIRT2 deacetylates H4K16 in certain cases while it deacetylates H3K18 in other cases, there may be different mechanisms to recruit SIRT2, which may affect the selectivity of SIRT2 for H3K18 versus H4K16.

4.4. Possibility of New Epigenetic Marks

Sirtuins were initially thought to be deacetylases, but recent evidence suggests that some of them can remove other acyl lysine modifications more efficiently.[34,44,45] Succinyl lysine was reported to occur on histones.[127] However, since SIRT5 is mainly in the mitochondria, whether histone succinylation is an epigenetic mark remains to be elucidated. A more interesting case is SIRT6. In vitro with peptide substrates, it is much more efficient at removing long chain fatty acyl groups. In vivo, the deacetylation of histone H3K9 and H3K56 by SIRT6 has been firmly demonstrated. The discrepancy between in vivo and in vitro may be partly explained by the report that SIRT6's deacetylase activity is increased on nucleosome substrates[363] or in the presence of free fatty acids (Table 3).[49] However, another possibility also needs to be further investigated, which is that histones lysine fatty acylation could be a new epigenetic mark and SIRT6 can regulate this mark. Given that histones have been shown to contain Cys palmitoylation,[364] Lys fatty acylation on histones is also possible.

4.5. Function of Different Histone Acetylation Sites

The available experimental evidence suggest that different mammalian sirtuins have preference for different histone acetylation sites. The transcriptional-silencing effect of SIRT2 is achieved through deacetylation of either H3K18 or H4K16, while the silencing effect of SIRT6 is achieved mainly through H3K9 deacetylation. Mechanistically, this is very interesting. Is deacetylation of a single site (e.g., H3K18) sufficient to suppress transcription or this is because only one site is acetylated in vivo at the relevant loci? Do different acetylation sites have different function (e.g., associated with target genes of different transcription factors)? The selectivity of sirtuins toward unique histone acetylation sites may provide a unique opportunity to address these questions. Addressing these

questions will provide important insights into the fundamental epigenetic mechanisms.

4.6. Drug Discovery Targeting Sirtuins and the Importance of Biochemical Assays

Since increasing lines of evidence provide significant support for the importance of sirtuins in many biological processes, there has been a broad interest in developing small molecules that regulate sirtuins. Sirtuin activators have attracted more interest given the importance of SIRT1 in mediating the beneficial effects of calorie restriction.[366] The natural polyphenol, resveratrol, was the first molecule shown to activate SIRT1[367] and extend the life span of *S. cerevisiae*,[367] *Drosophila*,[368] and *C. elegans*.[369] Later, large-scale screening identified more potent and specific SIRT1 activating compounds (STACs), including SRT1720, SRT2183, and SRT1460. More studies also showed that STACs are able to protect mice from high-fat-diet-induced metabolic disease.[370] However, studies on STACs still remain controversial. It was shown that the SIRT1-activating effect of STACs was only obvious when using an artificial p53-derived Fluo-de-Lys peptide, and the STACs did not lead to apparent SIRT1 activation with native p53 peptide lacking a fluorophore.[371,372] Later re-evaluation of the STACs revealed an assisted allosteric activation mechanism by which the hydrophobic fluorophore mimics the properties of endogenous substrates required for STACs-mediated SIRT1 activation. Analysis of several peptides with consensus motif carrying the corresponding properties revealed that their deacetylation by SIRT1 was enhanced by STACs, suggesting the STACs may work on specific SIRT1 substrates in vivo.[373] It should be pointed out that even though controversies exist, the concept of STACs is valid. More effort is needed to develop STACs that are chemically diverse but do not act by the assisted allosteric activation mechanism. These studies on the development of STACs also highlight the potential pitfalls of biochemical assays (especially high-throughput assays) and suggest that it is important to use alternative biochemical assays to validate results.

Sirtuin inhibitors have also been developed, and many of them have been shown to impact the progression of neurodegenerative disorders or to exhibit anticancer activity.[374,375] Also, specific sirtuin inhibitors can be excellent tools for in vivo studies of the physiological roles of sirtuins. However, the development of sirtuin inhibitors has been mainly limited to academia so far. The effects of sirtuins are extremely complex given the large number of biological substrates regulated by sirtuins and the multiple enzymatic activities possessed by a certain sirtuin. It remains challenging to determine the precise and disease-related targets of sirtuins and to fully understand the therapeutic potential of targeting sirtuins. As the disease relevance of sirtuins gets clearer, sirtuin inhibitor development may be increasingly taken up by pharmaceutical companies. The importance of biochemical assays is also reflected in the sirtuin inhibitor development. This is particularly relevant for sirtuins with weak deacetylase activities in vitro. For these sirtuins, the development of

inhibitors lags behind because there was no reliable assay to analyze the inhibition effects of compounds. As more efficient activities of these sirtuins are discovered, the inhibitor development for these sirtuins will speed up.

Given the fundamental questions and the therapeutic potentials of sirtuins mentioned above, we believe that research interest and activity in sirtuins will continue to rise and new exciting development will continue to occur in the next decade.

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Notes

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