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Updates on the epigenetic roles of sirtuins

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Sirtuins are a class of enzyme with NAD$^+$-dependent protein lysine deacylase activities. They were initially discovered to regulate transcription and life span via histone deacetylase activities. Later studies expanded their activities to other proteins and acyl lysine modifications. Through deacylating various substrate proteins, they regulate many biological processes, including transcription, DNA repair and genome stability, metabolism, and signal transduction. Here, we review recent understandings of the epigenetic functions (broadly defined to include transcriptional, post-transcriptional regulation, and DNA repair) of mammalian sirtuins. Because of the important functions of sirtuins, their own regulation is of great interest and is also discussed.

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Introduction

Sirtuins are a class of enzymes that have attracted much interest in the past decades. They were initially discovered as the regulators of aging and epigenetics. The founding member, yeast silencing information regulator 2 (Sir2), was found to be important for gene silencing and calorie restriction-induced life span extension in yeast [1,2]. While studying the gene silencing and longevity roles of Sir2, it was discovered that Sir2 is an NAD$^+$-dependent histone deacetylase [3,4], which stimulated great interest in this class of enzymes. In mammals, there are seven Sir2 homologs or sirtuins (SIRT1-7), found to regulate numerous substrate proteins and biological pathways. Interestingly, several sirtuins later were found to preferentially hydrolyze other acyl lysine modifications, such as succinyl and long-chain fatty acyl groups [5–9]. Although mammalian sirtuins have many other regulatory roles, such as those in metabolism and cell signaling, their function in epigenetics remains of great interest and is highlighted in many excellent review articles [10–13]. Here, we focus on the developments in the epigenetic roles of mammalian sirtuins in the last two years.

The epigenetic roles of SIRT1

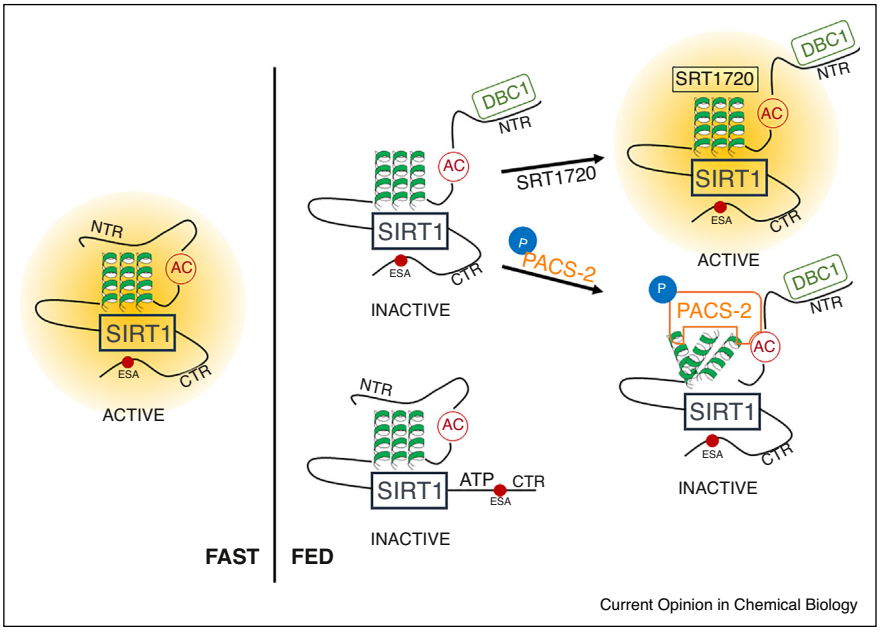
SIRT1 is the closest mammalian ortholog of the yeast Sir2 and plays a role in many physiological processes. Most are thought to be regulated through its lysine deacetylase activity, yet it was recently found that SIRT1 can efficiently remove fatty acyl chains from lysine residues *in vitro* [14,15]. It is the largest of the seven mammalian sirtuins with a conserved catalytic domain flanked by the extended N-terminus and C-terminus. While SIRT1 shuttles between the cytosol and the nucleus, most activities are done in the nucleus through deacetylation of histones and other transcriptional regulators [13]. In the past several years, many discoveries were made regarding its epigenetic role via deacetylation of histones and transcriptional regulators. More excitingly, new findings on SIRT1's role in DNA repair and RNA function and the regulatory mechanisms of SIRT1 activity provide novel and important insights and potential therapeutic strategies.

SIRT1 is tightly regulated to respond to cellular needs

Understanding how SIRT1 activity is fine-tuned to respond to specific stimuli is of great interest and importance. Recent studies demonstrate that this is through regulatory domains within SIRT1, post-translational modifications (PTMs), transcriptional control, posttranscriptional silencing, and protein–protein interactions.

SIRT1 deacetylase activity is regulated by the insulin sensor hidden in the disordered N-terminal region. In the presence of insulin, the sensor engages DBC1 and PACS-2 to inactivate SIRT1 [16,17]. An acidic cluster and a 3-helix bundle within the region are sequestered by a shield that is removed by DBC1, which in turn allows phosphorylated PACS-2 to bind the acidic cluster and one of the helices. This destabilizes the 3-helix bundle and inhibits SIRT1 catalysis and the subsequent PGC-1α and PPARα target gene transcription to promote diet-induced obesity (Figure 1). This work uncovered the mechanism of SIRT1 inhibition by DBC1 and PACS-2 and its role in insulin response [18••].

Figure 1



Regulation of SIRT1 via its N-terminal and C-terminal extensions by binding to small molecules or proteins. NTR = N-terminal region, CTR = C-terminal region, AC = acidic cluster, ESA = essential for SIRT1 activity region.

The C-terminal domain regulates SIRT1 via ATP. A 25-amino acid peptide in this domain can bind to ATP, causing compaction of the structure of SIRT1, decreasing its affinity for substrates and inhibiting the deacetylase function. Abolishing ATP-binding promotes SIRT1-mediated stress resistance in mice and inhibits adipogenesis in cultured MEFs. Interestingly, transferring this domain to other proteins also puts them under ATP regulation (Figure 1) [19••].

PTMs provide additional regulatory mechanisms of SIRT1 function. SIRT7 and SIRT1 cooperate in regulating adipogenesis through reversible SIRT1 acetylation and the consequent PPARγ transcription in mice. Cell culture, biochemical, and mouse studies provided unexpected evidences that SIRT7 antagonizes SIRT1 by preventing its autodeacetylation at K230 through a direct interaction to repress PPARγ [20].

SIRT1 activity is regulated by phosphorylation. During IL-6 stimulation, SIRT1 is phosphorylated by JAK1 at Tyr280 and Tyr301, which is required for SIRT1 interaction with its known deacetylation substrate STAT3 [21,22]. In diet-induced obese but not lean mice, SIRT1 is phosphorylated by CK2 at Ser164, which inhibits its nucleus localization and deacetylase activity. Strongly elevated levels of CK2 and SIRT1 phosphorylation at Ser164 were also observed in liver samples from patients with nonalcoholic fatty liver disease [23]. Furthermore, under oxidative stress SIRT1 is activated by dephosphorylation at Ser47 mediated by the GADD34/PP1α complex [24].

O-linked *N*-acetyl-β-D-glucosamine (*O*-GlcNAc) is another PTM recently shown to regulate SIRT1 activity. *O*-GlcNAc transferase modifies SIRT1 on Ser549, increasing its deacetylase activity. Under genotoxic stress *O*-GlcNAc-modified SIRT1 but not the S549A mutant robustly deacetylates p53 to promote cell survival [25]. Activation of SIRT1 by sulfhydration enhances its zinc-binding and stability to alleviate atherosclerotic plaque [26].

SIRT1 function in epigenetics is also affected by miRNA and lncRNA targeting, transcriptional regulation by HIF1α, SUV39H and interaction with BRG1 (Table 1) [27–44]. These reports portray the intricate regulation network of SIRT1 enzymatic function and its importance for normal physiology and diseases.

SIRT1 controls transcription through epigenetic regulators

SIRT1 is known to regulate transcription by deacetylating histones and other epigenetic regulators. In the past two years, new transcription factors (TFs) have been identified as deacetylation substrates of SIRT1 and novel roles have been found for the regulation of TFs and histone marks that are established SIRT1 substrates. Although the regulation of TFs and histone marks is one of the most prominent epigenetic roles of SIRT1,

Table 1

miRNA and lncRNA regulating SIRT1 levels

Name	Effect on SIRT1 levels	Functions	References
miR-199a-5p	Decrease	Regulates the pathogenesis of intrauterine growth restriction	[27]
miR-361-5p	Decrease	Promotes hepatic triglyceride accumulation and insulin sensitivity during hepatosteatosis	[28]
miR-34a	Decrease	1) Suppresses proliferation and apoptosis of gastric cancer 2) Regulates pro-apoptotic caspase-3/7 activity and p53 levels in heart cells 3) Downregulates plasma and hepatic Fgf21 during obesity and insulin resistance	[29,32,35]
miR-29b	Decrease	Reverses oxaliplatin-resistance in colorectal cancer by enhancing ROS and JNK phosphorylation to induce apoptosis	[30]
miR-30a	Decrease	Suppresses lung cancer progression	[31]
miR-221	Decrease	Promotes white adipose tissue inflammation and insulin-resistance	[33]
miR-204	Decrease	In prostate cancer indices mitochondrial apoptosis through upregulation of Noxa and Puma via acetylated p53	[34]
miR-181a	Decrease	Induces gastric cancer apoptosis by increasing FoxO1 acetylation during oxidative stress	[36]
lncRNA NEAT1	Increase	Promotes cell proliferation and metastasis in colorectal cancer by competing with miR-34a leading to upregulation of Wnt/βcatenin signaling	[37]
lncRNA MALAT1	Increase	In high glucose represses SIRT1 transcription by interacting with FoxO1 to induce HK-2 cell injury	[38]
lncRNA-PRLB	Increase	Promotes breast cancer by targeting miR-4766-5p to increase SIRT1 levels	[39]
LncRNA HNF1A-AS1	Increase	Promotes colon cancer metastasis by increasing SIRT1 levels through targeting miR-34a to induce noncanonical Wnt signaling	[40]
LncRNA HULC	Increase	Inhibits SIRT1 degradation to promote protective autophagy in hepatocellular carcinoma	[41]

since it has been extensively reviewed, we provide only a brief additional update in Table 2 without further discussion.

However, it worth mentioning that SIRT1-catalyzed histone deacetylation often cross-talks with other epigenetic modifications. For example, in MLL-AF9 driven leukemia cells with DOT1L inactivation, SIRT1 deacetylates H3K9 of MLL target genes allowing subsequent H3K9 dimethylation by SUV39H1 to increase chromatin compaction and gene silencing. The combination of SIRT1 activator SRT1720 and DOT1L inhibitor EP24777 was able to suppress MLL-AF9 driven tumor formation in mice, suggesting a potential therapeutic approach against hard-to-treat MLL-rearranged leukemia [45]. SIRT1 has also been reported to associate with KDM2B, an eraser of H3K79 methylation, at gene promoters. The subsequent H4K16 deacetylation by SIRT1 promotes transcriptional repression [46].

SIRT1 can promote or inhibit DNA repair

DNA lesions caused by UV radiation or genotoxins can block transcription leading to apoptosis. SIRT1 is known to regulate the homologous recombination (HR) repair machinery proteins NBS1 and Rad51 by deacetylation and recruitment to DNA damage sites [47,48]. Inactivation of SIRT1 impairs HR repair and enhances lung cancer apoptosis during inhibition of WEE1, a kinase regulator of G2/M checkpoint [49]. In response to the UV-induced DNA damage, SIRT1 deacetylates XPA allowing its phosphorylation by ATR to alleviate the damage via nucleotide excision repair (NER) [50,51]. In addition, the regulator of DNA metabolism RPA1 is acetylated on K163 by PCAF acetyltransferase upon UV-induced DNA damage, which enhances the RPA1 interaction with XPA to promote repair. SIRT1 and HDAC6 erase the acetylation to release XPA from the original DNA damage site at the end of the repair process [52]. SIRT1 also deacetylates BRCA1 to inhibit the intra-S check point and promote DNA replication and cell growth [53,54]. Furthermore, a nucleosome assembly protein TSPY-Like 2 inhibits SIRT1 during DNA damage to induce apoptosis through hyperacetylated p53 [13,55]. These studies underscore the different roles of SIRT1 in DNA repair pathways and stages.

SIRT1 regulates RNA metabolism

SIRT1 has recently emerged as an RNA regulator. In HPV-infected cervical cancer cells, SIRT1 suppressed the levels of AIM2 inflammasome gene, allowing the cells to escape the immune response. SIRT1 knockdown caused pyroptosis and promoted RelB-dependent AIM2 transcription. Unexpectedly, SIRT1 depletion increased RelB mRNA, but not pre-mRNA, and elevated AIM2

Table 2

Recently reported deacetylation substrates of Sirtuins

Type	Substrate	Full name	Modification sites	Functions	Reference
SIRT1					
Transcription factors	PRRX1	Paired related homeobox 1	K160	Upregulates KLF4 to promote cell stemness and migration through LDH1 upregulation	[122]
	KLF4	Kruppel-like factor 4	Unknown	Regulates the ovarian cancer cell invasion by inducing the expression of Claudin-5	[123]
	NFATc1	Nuclear factor of activated T-cells, cytoplasmic 1	Unknown	Polarization and recruitment of adipose tissue macrophages	[124]
	C/EBPα	CCAAT/enhancer-binding protein alpha	K159, K298	Increases mitochondrial respiration by upregulating mitochondrial genes	[125]
Others	TET2	Tet methylcytosine dioxygenase 2	K1468, K1472, K1473, K1478	Activates TET2 to ameliorate myelodysplastic syndrome	[126•]
	MDM2	E3 ubiquitin ligase murine double minute 2	K182, K185	Promotes self-ubiquitination and degradation of MDM2, which stabilizes p53 to promote apoptosis in osteosarcoma	[127]
	DNMT3l	DNA (cytosine-5)-methyltransferase 3-like	Unknown	Destabilizes DNMT3l protein by direct deacetylation to promote ESCs differentiation	[128]
	RPA1	Replication protein A1	K163	Decreases interaction with XPA during DNA repair	[52]
	PABP1	Poly(A)-binding protein 1	K95	Retains of PABP1 and mRNA in the nucleus inhibiting protein translation	[57•]
	9G8	SR protein 9G8	K24	Promotes the inclusion of tau exon 10	[58]
SIRT2					
Histone	H3	Histone H3	K18	Translocates SIRT2 into nucleus to deacetylate H3K18 to reprogram transcription, when *Listeria monocytogenes* infected	[75]
Transcription factors	NFAT	Nuclear factor of activated T-cells	Unknown	Destabilizes NFATc2 and suppresses its nuclear localization, resulting in decreased transcription activity to cardiac homeostasis	[129]
	NRF2	Nuclear factor erythroid-derived 2–related factor 2	K506, K508	Reduces NRF2 levels, to reduce ferroportin 1 expression and decrease iron export	[130]
	Slug	Slug	K116	Stabilizes slug	[73]
	JNK	c-Jun NH2-terminal kinases	K153	Enhances ATP binding and enzymatic activity of JNK towards c-Jun; favors the phosphorylation of JNK by MKK4	[131]
	p73	Tumor suppressor p73	K620, K623, K627	Suppresses its transcriptional activity, critical for tumorigenicity of glioblastoma cells	[74]
Metabolism enzyme	ALDA	Aldolase	K322	Suppresses glycolytic enzyme activities, and regulates metabolic reprogramming during induced pluripotency	[59]
	ENO1	Enolase	Unknown	Suppresses glycolytic enzyme activities, and regulates metabolic reprogramming during induced pluripotency	[59]
	PGK1	Phosphoglycerate kinase 1	Unknown	Suppresses glycolytic enzyme activities, and regulates metabolic reprogramming during induced pluripotency	[59]
	GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Unknown	Suppresses glycolytic enzyme activities, and regulates metabolic reprogramming during induced pluripotency	[59]
	PKM2	M2 isoform of pyruvate kinase	K305	Induces PKM2 activity by promoting tetramerization to the active enzymatic form	[132]

Table 2 (*Continued*)

Type	Substrate	Full name	Modification sites	Functions	Reference
Others	ANKLE2	Ankyrin and LEM domain-containing protein 2	K302	Promotes ANKLE2 phosphorylation and nuclear envelope reassembly	[133]
	ATRIP	ATR-interacting protein	K32	Drives ATRIP phopshorylation and accumulation to DNA damage sites in response to replication stress	[76]
	GSK3	Glycogen synthase kinase 3	K246/K183	Enhances its binding to ATP	[134]
SIRT6					
Histone	H3	Histone H3	K9	Silences Notch1/4, suppresses Notch signaling in podocytes	[86]
	H3	Histone H3	K9	Allows ADP-ribosylation in response to DNA damage	[98••]
	H3	Histone H3	K56	Silences IGFBP2, activates AKT signaling in melanoma cell	[87]
	H3	Histone H3	K56	Promotes ATF4 destabilization from target genes	[92]
	H3	Histone H3	K9, K56	Silences LIN28b, suppresses let-7 target genes in pancreatic cancer	[88•]
	H3	Histone H3	K9, K56	Transcriptionally suppresses long non-coding RNA H19	[89]
	H3	Histone H3	K9, K56	Silences pluripotency genes in embryonic stem cells	[90]
Transcription factors	p53	Tumor suppressor 53	K381	Destabilizes p53	[94•]
	PKM2	Pyruvate kinase M2 (nuclear)	K433	Leads to nuclear export of nuclear PKM2, suppressing its transcription activity in hepatocellular carcinoma	[95]
Others	TRF2	Telomere repeat binding factor 2	K176, K179, K190	Destabilizes TRF2 in response to DNA damage	[97]
SIRT7					
Transcription factors	SP7/OSX	Osterix	K368	Promotes its transactivation activity in bone formation	[135]
Others	DDX21	DEAD-box RNA helicase	K18, K137, K600	Augments helicase activity and overcomes R-loop-mediated stalling of RNA polymerases, safeguards genome stability	[117]

inflammasome-containing exosomes that propagated pyroptosis to neighboring naïve cells. These findings hint to an existing role of SIRT1 in mRNA stability and exosome biogenesis that require further mechanistic delineation [56•].

SIRT1 also protects mRNA transport. During energy starvation, SIRT1 is phosphorylated on T530, allowing SIRT1 to bind and deacetylate K95 of PABP1, a poly(A)-binding protein. This leads to the retention of PABP1 and mRNA in the nucleus, inhibiting protein translation and cell proliferation to conserve energy consumption [57•].

Another RNA regulatory role of SIRT1 is in the alternative splicing of tau. SIRT1 deacetylates K24 of the splicing factor 9G8 to promote the inclusion of tau exon 10. SIRT1 or 9G8 K24R overexpression in HEK293T cells or resveratrol treatment of Htau mice with high levels of tau promote exon 10 inclusion. Of interest is to determine how 9G8 is affected by acetylation to regulate pre-mRNA splicing [58]. These reports open new exciting avenues for understanding the SRT1 function in RNA regulation.

The epigenetic roles of SIRT2

SIRT2 resides mainly in cytoplasm with highly dynamic nucleo-cytoplasmic shuttling and is connected to a wide range of physiological processes, like cell cycle, genome stability, metabolism, and aging. While half of the functions involves deacetylation of cytosolic proteins, including metabolic enzymes [59], SIRT2 regulates many epigenetics processes, including deacetylation of histone marks (H4K16ac [60] and H3K56ac [61]), transcription factors (FoxO1 [62], FoxO3 [63], HIF1α [64]), and chromatin modifying enzymes (p300 [65]).

Recent progress in SIRT2 study highlights the diverse enzymatic activities of SIRT2 and the potential impacts in epigenetics, through its accommodation of other acyl lysine modifications. SIRT2 removes lysine fatty acylation on K-Ras4a, its first identified defatty-acylation substrate [66,67]. The deacylation leads to subcellular redistribution of K-Ras4a and promotes cellular transformation. SIRT2 also removes lysine γ-oxo-nonanoylation [68,69], a histone modification derived from lipid peroxidation products, and lysine benzoylation [70].

SIRT2's epigenetic role has been connected to cancer. Early reports showed that SIRT2 regulates cell cycle checkpoint and acts as a weak tumor suppressor

[60,71]. Recently, several new transcription factors were reported to be regulated by SIRT2 and inhibiting SIRT2 confers anticancer activity. Selective inhibition of SIRT2 using a thiomyristoyl-lysine small molecule, TM, promotes c-Myc degradation and exerts a broad anticancer effect in various human cancer cells and mouse models of breast cancer [72••]. This is achieved via an epigenetic mechanism as the mRNA level of several E3 ubiquitin ligases of c-Myc is upregulated by SIRT2 inhibition. SIRT2 deacetylates and stabilizes Slug [73], a transcription factor, important for epithelial to mesenchymal transition (EMT). More recently, in an RNA interference screen in a glioblastoma model, SIRT2 was found to suppress glioblastoma cells through deacetylating and inhibiting of tumor suppressor p73 [74].

Recent reports highlight the function of SIRT2 in stress response, which also involves its epigenetic function. SIRT2 is translocated to the nucleus under *Listeria monocytogenes* infection and targeted to chromatin to deacetylate H3K18 and reprogram gene expression [75]. Interestingly, SIRT2 is detrimental for controlling the infection. SIRT2 deacetylates K32 of ATR interacting protein (ATRIP) in response to hydroxyurea-induced replication stress, which promotes ATR activation and accumulation to DNA damage sites and binds to replication protein A-coated single-stranded DNA [76]. SIRT2 also deacetylates and activates JNK, promoting oxidative stress-induced cell death [77].

SIRT6 in epigenetic regulation

SIRT6 is important in DNA damage response, metabolism, aging, and cancer. SIRT6, as a tumor suppressor, represses HIF1α [78] and c-Myc [79]. SIRT6 knockout mice have severe metabolic defects, genome instability, and premature aging, while Sirt6-transgenic mice have a longer lifespan [80]. This advocates that SIRT6 has a clear longevity role. Those findings draw people's attention and efforts to develop SIRT6 activator to study its cellular functions and explore the therapeutic potential.

SIRT6 and histone marks

SIRT6 deacetylates H3K9 [81] and H3K56 [82] to suppress transcription. As a histone deacetylase, SIRT6 represses transcription mediated by many transcription factors, including HIF1α [78], cMyc [79], NF-kB [83], c-Jun [84], and FoxO3 [85].

In the past two years, more physiological functions of SIRT6 as a histone deacetylase are explored. Sirt6 has pleiotropic protective actions in podocytes, through deacetylating H3K9 at the promoter region of Notch1 and Notch4 and suppressing Notch signaling [86]. SIRT6 haploinsufficiency confers melanoma cell resistance to MAPK inhibitors, through increased H3K56 acetylation at IGFBP2 locus, which transcriptionally activates IGF-1 receptor and downstream AKT signaling [87].

SIRT6 also regulates the expression of non-coding RNAs. Loss of SIRT6 increases the protein Lin28b through promoter histone hyperacetylation, leading to induction of downstream let-7 target genes [88•]. This epigenetic program defines a distinct subset (30%–40%) of human pancreatic ductal adenocarcinoma with a poor prognosis. SIRT6-null cynomolgus monkeys died hours after birth and exhibited severe prenatal developmental retardation [89]. Mechanistically, SIRT6 deficiency results in imprinting control region histone hyperacetylation and, therefore, activating long non-coding RNA H19, a developmental repressor.

SIRT6 itself is also regulated by long non-coding RNA. LncPRESS1, a p53-regulated LncRNA, can sequester SIRT6 from chromatin association, leading to high levels of histone acetylation at promoters of pluripotency genes, which maintains human embryonic stem cells pluripotency [90].

SIRT6 and transcription factors

SIRT6-regulated transcription factors can silence or activate transcription. SIRT6 in adipose tissue is cold-inducible and recruits phospho-ATF2 to activate PGC-1α gene expression and promote thermogenesis [91]. SIRT6 is a co-repressor of ATF4, as deacetylation of H3K56 promotes ATF4 destabilization from target genes. In autophagy deficient cells, glutamine depletion induces SIRT6 dissociation from ATF4 and induces amino acid transporter expression [92]. Palmitate treatment increases the levels and interaction of SIRT6 and p53, activating p53 targeted genes involved in cardiolipin synthesis [93]. As such, SIRT6 serves as a co-activator of p53. Interestingly, a different SIRT6–p53 relationship has also been reported. Haploinsufficiency of p53 in Sirt6-deficient mice rescues several age-related phenotypes, as SIRT6 deacetylates and destabilizes p53 [94•]. This could partially explain the extended lifespan of SIRT6 transgenic mice.

SIRT6 deacetylates nuclear PKM2, which leads to nuclear export of PKM2, suppressing its nonmetabolic oncogenic functions in transcriptional regulation. This nuclear export contributes to SIRT6 tumor-suppressor functions *in vivo* [95].

SIRT6 and DNA damage repair

SIRT6 facilitates Ku80/DNA–PKcs interaction and promotes DNA–PKcs phosphorylation, leading to efficient non-homologous end joining, which is important in aging and pluripotency [96]. Under DNA damage condition, SIRT6 deacetylates and destabilizes telomere repeat binding factor 2, which is involved in telomere maintenance and DNA damage response [97].

It was recently shown that DNA-damage-induced H3S10 ADP-ribosylation is blocked by H3K9 acetylation [98••]. This interesting crosstalk explains the previous finding that SIRT6 often migrates to the DNA damage sites and

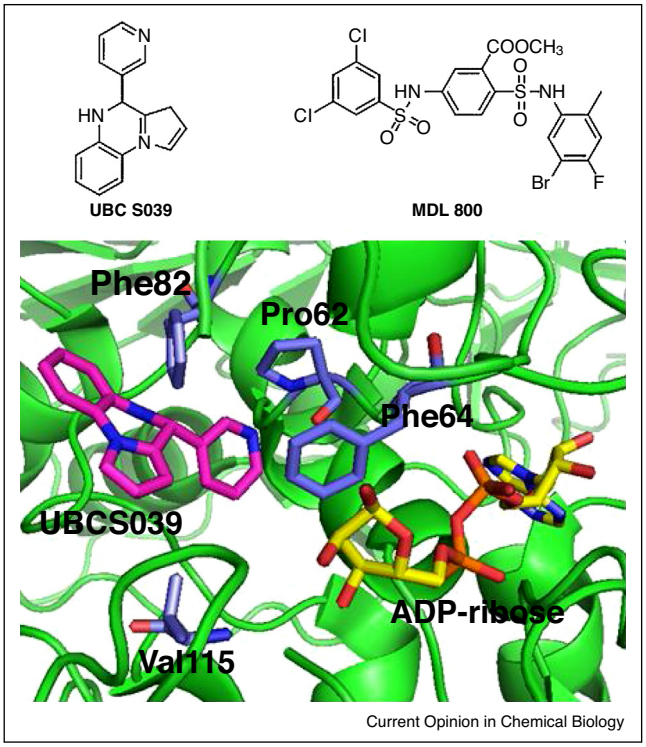
activates PARP1, a known DNA repair regulator. This work explains how a cell responds to DNA damage in the pre-existing and elaborate chromatin landscape.

The contributions of different activities of SIRT6 in epigenetics

While the deacetylase activity of SIRT6 is clearly important for epigenetic regulation, SIRT6 can also efficiently remove long-chain fatty acyl groups from TNFα and R-Ras2 [6,99]. Whether the defatty-acylase activity also has epigenetic roles is thus of great interest. Using a SIRT6 G60A mutant that abolishes the deacetylase activity but retains demyristoylase function, it was demonstrated that most of the transcriptional regulation by SIRT6 may come from its deacetylase activity [100].

The multiple activities also enable the development of small molecule modulators of SIRT6 that only affect one of the activities. Free fatty acids were found to activate SIRT6's deacetylase activity but not demyristoylase activity [14]. A pyrrolo[1,2-a]quinoxaline-based compound, UBCS039, which binds to the fatty acyl pocket of SIRT6 (Figure 2), was reported to promote the deacetylation activity of SIRT6 without affecting the demyristoylation activity [101]. UBCS039-treated H1299 cells showed lower acetylation level of H3K9ac and H3K56ac and autophagy-induced cell death [102]. In 2018, MDL-800 was found to activate SIRT6's deacetylase activity selectively. MDL-800 decreased acetyl H3K9 and H3K56 in cells, two major SIRT6 substrates. In a mouse tumor xenograft model, MDL-800 decreased tumor volumes [103]. These SIRT6 activators, given that they only activate the deacetylase activity of SIRT6, may prove to be useful probes for studying the role of SIRT6's deacetylation activity in epigenetics in combination with the G60A mutant of SIRT6.

Figure 2



The structures of two synthetic small molecule activators of SIRT6's deacetylase activities. One of them, UBCS039 is shown to bind the fatty acyl pocket of SIRT6.

SIRT7 in epigenetic regulation

SIRT7 resides in the nucleus but concentrates in nucleoli and is known to deacetylate H3K18 [104]. It was also reported to desuccinylate histones [105], but the *in vitro* biochemical evidence is not very strong. The *in vitro* activity of SIRT7 is weak but can be enhanced dramatically by double stranded DNA and different RNA species [106•,107].

SIRT7 is a unique sirtuin as it regulates transcription mediated by all three nuclear RNA polymerases. Proteomics and enzymology studies revealed that SIRT7 regulates Pol I activity and ribosome biogenesis [108–110], Pol II mRNA transcription and metabolic stress response [111–114], and potential regulation of Pol III transcription [115].

The role of SIRT7 in genome stability was recently discovered. SIRT7 maintains rDNA stability and guards against cellular senescence through regulating SNF2H, a component of the nucleolar heterochromatin-silencing complex [116••]. SIRT7 deficiency leads to defective rDNA-heterochromatin silencing, causing rDNA instability and cell senescence. SIRT7 also safeguards genome stability through deacetylating and augmenting DDX21, an RNA helicase that unwinds aberrant R loop and maintains genome stability [117].

Concluding remarks

While many studies continue to unveil the epigenetic roles of sirtuins as deacetylases for histones and transcriptional regulators, the studies described above raise new questions and open new research opportunities. The emerging RNA regulatory role of SIRT1 requires more mechanistic delineation. Whether sirtuin's lysine de-fatty acylase activity has epigenetic roles remains to be determined. Identification of protein substrates of this activity and its downstream effects can shed light on this largely ignored area of study.

It is amazing that recent studies revealed many additional substrates for the seven mammalian sirtuins. This highlights the important biological functions of sirtuins, yet calls for a deeper understanding to simplify the complexity. In this regard, it is becoming increasingly important to understand how the sirtuins themselves are

regulated, which will provide key insights to sort out the complexity associated with so many different substrates and functions. SIRT1, the most well studied mammalian sirtuins, is again at the leading edge. As discussed above, SIRT1 is the target of regulation by cellular stimuli necessary to meet specific demands. Understanding how other sirtuins are regulated will similarly provide important insights.

At present, the mitochondrial sirtuins, SIRT3-5, seem to have limited direct effects in epigenetics due to their cellular localization. Nevertheless, their nuclear localization should not be completely disregarded. In fact, SIRT3 was reported to localize in the nucleus and regulate histone acetylation [118] and crotonylation [119], but the nuclear localization of SIRT3 was controversial [120]. Histones were known to be succinylated and the first histone H3 succinyltransferase was recently discovered [121]. SIRT5, the only well accepted lysine desuccinylase, could possibly regulate such histone modification and thus have direct epigenetic roles. Future studies should further clarify the epigenetic functions of SIRT3-5.

Sirtuins are increasingly recognized as deacylases instead of deacetylases. However, the epigenetic functions reported have largely attributed to the deacetylase activity. Whether other activities regulate epigenetics is, therefore, of great interest. It is possible that other acyl lysine modifications are also epigenetic marks. Alternatively, other deacylation functions of sirtuins are mainly confined to locations outside of the nucleus.

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- • of special interest
- •• of outstanding interest

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