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Understanding the Function of Mammalian Sirtuins and Protein Lysine Acylation

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Abstract

Protein lysine acetylation is an important posttranslational modification that regulates numerous biological processes. Targeting lysine acetylation regulatory factors, such as acetyltransferases, deacetylases, and acetyl-lysine recognition domains, has been shown to have potential for treating human diseases, including cancer and neurological diseases. Over the past decade, many other acyl-lysine modifications, such as succinylation, crotonylation, and long-chain fatty acylation, have also been investigated and shown to have interesting biological functions. Here, we provide an overview of the functions of different acyl-lysine modifications in mammals. We focus on lysine acetylation as it is well characterized, and principles learned from acetylation are useful for understanding the functions of other lysine acylations. We pay special attention to the sirtuins, given that the study of sirtuins has provided a great deal of information about the functions of lysine acylation. We emphasize the regulation of sirtuins to illustrate that their regulation enables cells to respond to various signals and stresses.

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INTRODUCTION

Protein lysine acetylation has been recognized for over 50 years. It was initially observed on histone proteins and plays important roles in regulating transcription (1–3). Later studies revealed that many other proteins are also regulated by lysine acetylation, most notably transcription factors and metabolic enzymes. Proteomic studies have revealed over 2,000 proteins that contain lysine acetylation and are potentially regulated by this posttranslational modification (PTM) (4, 5). Therefore, acetylation plays important regulatory roles in biology, similar to those of phosphorylation and ubiquitination.

In the past decade or so, many new acyl-lysine modifications, including butyrylation, crotonylation, succinylation, malonylation, glutarylation, hydroxyisobutyrylation, 2-hydroxybutyrylation, and long-chain fatty acylation (**Figure 1**), have been reported to occur on proteins. The functional significance of these new acyl-lysine modifications is just beginning to be elucidated.

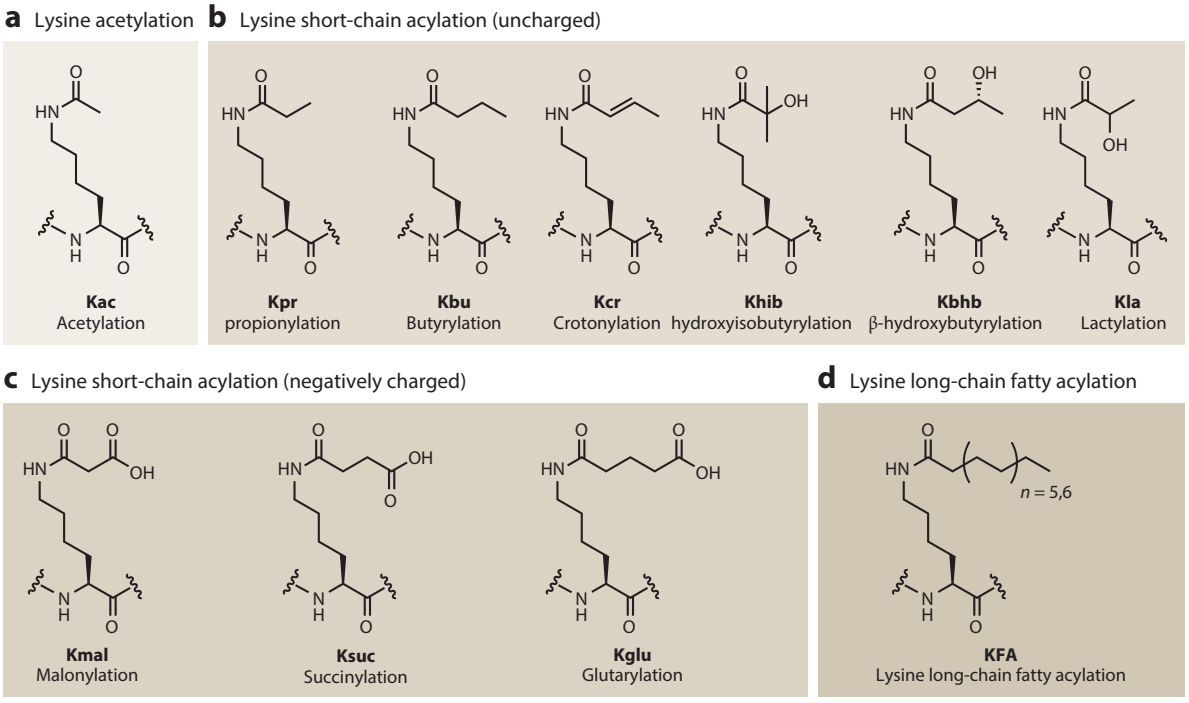

Figure 1

Structures of acyl-lysine modifications including acetylation, propionylation, butyrylation, crotonylation, hydroxyisobutyrylation, β-hydroxybutyrylation, lactylation, malonylation, succinylation, glutarylation, and long-chain fatty acylation.

In this article, we provide a review of the functional significance of various known acyl-lysine modifications, with a focus on acetylation, which is the most well understood and enables us to distill some principles that help elucidate the functions of other acyl modifications. We pay special attention to sirtuins, the enzymes that remove acyl-lysine modifications, as the function of the acyl-lysine modifications in many cases has been revealed through the study of sirtuins. This review is not meant to be comprehensive, but instead, we focus on mammalian systems and emphasize important points to provide some guidelines that would be helpful for future studies.

WRITERS, READERS, AND ERASERS OF LYSINE ACETYLATION

Our understanding of the function of acetylation has been propelled by several important findings, including the discovery of enzymes that add or remove the acetyl group, which historically have been called histone acetyltransferases (HATs) (6, 7) and histone deacetylases (HDACs), respectively (for a list of HATs and HDACs mentioned in this review, see **Table 1**) (8, 9), as well as proteins that bind to acetyl-lysine (10). A recent review nicely summarized these major historical discoveries (11). In the epigenetic field, these molecules are often referred to in terms of their role within the histone code hypothesis: HATs are known as writers of acetylation, HDACs as erasers of acetylation, and proteins that bind acetyl-lysine as readers of acetyl-lysine (for a list of readers mentioned in this review and their binding specificities, see **Table 2**) (12).

HATs invariably use acetyl-Coenzyme A (acetyl-CoA), a major metabolite involved in many metabolic pathways, to acetylate substrate proteins. They are classified into a few different families

Table 1 Activity and specificity of lysine acylation modulating enzymes

Enzyme function	Enzyme family	Enzyme	Lysine acylation activity										Enzyme specificity	Reference(s)
			Kac	Kpr	Kbu	Kcr	Khib	Kbhb	Kmal	Ksuc	Kglu	KFA		
Writer	p300/CBP	p300/CBP	√	√	√	√	√	√	ND	ND	ND	ND	Kac:Kpr:Kbu:Kcr 1:0.33:0.02:0.02[a]	13, 327, 328
	MYST	MOZ	√	√	NR	NR	NR	NR	NR	NR	NR	NR	Kac:Kpr 1:1.0[b]	14
		MOF	√	√	NR	√	NR	NR	NR	NR	NR	NR	Kac:Kpr 1:0.8[b]	14, 315
		HBO1	√	√	NR	NR	NR	NR	NR	NR	NR	NR	Kac:Kpr 1:1.0[b]	14
		Tip60	√	√	NR	NR	√	NR	NR	NR	NR	NR	Kac:Kpr 1:0.13[b]	14, 329
	GNAT	GCN5	√	√	√	√	NR	NR	NR	√	√	NR	Kac:Kpr:Kbu 1:0.008:002[c]	15, 282, 316
		NMT1/2	NR	NR	NR	NR	NR	NR	NR	NR	NR	√	Not determined	16
Eraser	Class I HDACs	HDAC1	√	NR[d]	NR	√	√	NR	NR	NR	NR	NR	Kac:Kcr 1:0.4[e]	17, 310, 329
		HDAC2	√	NR[d]	NR	√	√	NR	NR	NR	NR	NR	Not determined	17, 310, 329
		HDAC3	√	NR[d]	NR	√	√	NR	NR	NR	NR	NR	Not determined	17, 310, 329
		HDAC8	√	NR[d]	√	√	ND	NR	NR	NR	NR	√	Kac:Kbu:Kmyr:Kpal 1:0.2:2:0.5[f]	18, 329
	Class IV HDACs	HDAC11	√	NR[d]	ND	NR	ND	NR	NR	ND	ND	√	Kac:Kmyr 1:1.54 × 10^4[f]	19, 329
	Class III HDACs	SIRT1	√	√	√	√	ND	√	ND	ND	ND	√	Kac:Kmyr:Kpal 1:1.2:1.2[g]	20, 337, 338
		SIRT2	√	√	√	√	ND	√	ND	ND	ND	√	Kac:Kmy 1:5.1[f]	21, 338
		SIRT3	√	√	√	√	√	√	ND	ND	ND	√	Kac:Kmyr:Kpal 1:2.0:2.0[g]	20, 338
		SIRT5	√	NR	√	ND	√	√	√	√	√	NR	Kac:Kmal:Ksuc 1:782:551[f]	22, 338
		SIRT6	√	√	√	√	ND	ND	ND	ND	ND	√	Kac:Kbu:Kmyr:Kpal 1:2:292:625[f]	23, 338
		SIRT7	√	ND	ND	ND	ND	ND	ND	√	ND	√	Kac:Kmyr 1:2.6[h]	24, 279, 338

[a]Based on the rates of catalysis measured using the indicated acyl-CoA cofactors (250 μM) and N-terminal histone H3 peptide (100 μM). p300 was at a concentration of 0.2 μM.
[b]Based on the k_{cat}/K_m values measured by fluorogenic assay with respect to the indicated acyl-CoA under the initial velocity condition.
[c]Based on the rate of catalysis measured using the indicated acyl-CoA cofactors (500 μM) and N-terminal histone H3 peptide (250 μM). GCN5 was at a concentration of 10 μM.
[d]Treatment with an HDAC inhibitor mixture induced lysine propionylation on p53 (337).
[e]Based on the k_{cat}/K_m values measured by dot-blot assay with respect to the indicated acyl-H3 under the initial velocity condition.
[f]Based on the k_{cat}/K_m values measured by HPLC assay with respect to H3K9 peptide.
[g]Based on the k_{cat}/K_m values measured by FL assay with respect to H3K9 peptide.
[h]Based on the k_{cat}/K_m values measured by HPLC assay in the presence of RNA, with respect to H3K18 peptide.
Abbreviations: FL, fluorescence; HDAC, histone deacetylase; HPLC, high-performance liquid chromatography; Kac, lysine acetylation; Kbhb, lysine β-hydroxybutyrylation; Kbu, lysine butyrylation; Kcr, lysine crotonylation; KFA, long-chain fatty acylation; Kglu, lysine glutarylation; Khib, lysine 2-hydroxyisobutyrylation; Kmal, lysine malonylation; Kmyr, lysine myristoylation; Kpr, lysine propionylation; Ksuc, lysine succinylation; ND, not detected (or activity orders of magnitude lower than its major activity); NMT, N-terminal glycine myristoyltransferase; NR, not reported.

Table 2 Specificity of lysine acylation readers

Reader domain	Reader enzyme	Substrate modification site	Binding specificity KD (μM)[a]							Reference
			Kac	Kpr	Kbu	Kcr	Khib	Kbhb	Ksuc	
Bromodomain	$BRD4_{BrD1}$	H4K8	270	>1,200	>3,000	>3,000	NR	NR	NR	25
	BAZ2A	H3K14	20	167	ND	ND	NR	NR	NR	25
	$BRD3_{BrD2}$	H3K18	45	164	1,690	ND	NR	NR	NR	25
YEATS domain	AF9	H3K9	5.0	2.7	3.7	2.1	ND	NR	500	25
	Taf14	H3K9	NR	NR	NR	9.5	NR	NR	NR	26
	YEATS2	H3K27	226.2	148.4	123.6	31.7	141.4	NR	NR	27
Double PHD finger domain	$DPF2_{DPF}$	H3K14	0.66	0.32	0.15	0.085	47.8	0.93	57.1	28
	MOZ_{DPF}	H3K14	22.9	10.8	10.0	5.8	132	110	ND	28

[a]Based on the kcal/mol values measured by isothermal titration calorimetry assay.

Abbreviations: Kac, lysine acetylation; Kbhb, lysine β-hydroxybutyrylation; Kbu, lysine butyrylation; Kcr, lysine crotonylation; Khib, lysine 2-hydroxyisobutyrylation; Kpr, lysine propionylation; Ksuc, lysine succinylation; NR, not reported; ND, not detected.

based on sequence and structural similarity, including HAT1, Gcn5/PCAF, MYST (MOZ, Ybf2, Sas2, and Tip60), p300/CBP, and Rtt109 (29). Most HATs have multiple substrates, except for the α-tubulin acetyltransferase ATAT1, which is specific for α-tubulin (29, 30). All HATs share a structurally conserved catalytic-core region for binding acetyl-CoA, yet this core region has very little sequence homology among different subfamilies (29).

HDACs consist of two different families of enzymes, the zinc-dependent HDAC1–11 proteins and the NAD^+-dependent sirtuins, which are homologs of the yeast Sir2 (silencing information regulator 2) protein. Many zinc-dependent HDACs are multidomain proteins and form large protein complexes with other proteins (31). Most sirtuins, in contrast, are single-domain proteins with only small terminal extensions flanking the catalytic domain (32).

The 11 zinc-dependent HDACs are further classified into class I (HDAC1, 2, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and 10), and class IV (HDAC11). The zinc-dependent HDACs bind zinc with conserved aspartate and histidine residues, and the zinc serves to activate a water molecule to hydrolyze the acetyl-lysine. However, HDAC10 (33) and HDAC11 (19, 34, 35) do not have deacetylase activity in vitro but instead hydrolyze *N*-acetylspermidine and long-chain fatty acyl–lysine, respectively. Similarly, class IIa HDACs have no detectable deacetylation activity in vitro but instead can remove trifluoroacetyl-lysine (36). Whether their in vivo functions are through deacetylation or some other yet-to-be-discovered activity remains unclear.

The sirtuins use NAD^+ as a cosubstrate to catalyze protein lysine deacetylation (37). In each deacetylation reaction, one molecule of NAD^+ is consumed and converted to 2′-*O*-acetyl ADP-ribose and nicotinamide (**Figure 2**) (38). The fact that NAD^+ is an obligate cosubstrate of sirtuins and the observation that some sirtuins are responsible for the beneficial effects of caloric restriction raise the hypothesis that sirtuins sense the level of NAD^+ in cells to catalyze protein lysine deacetylation (9). This is likely to depend on the K_m value of different sirtuins for NAD^+ and the physiological concentration of NAD^+. This view has also inspired many researchers to explore the supplementation of NAD^+ biosynthesis precursors to achieve health benefits (39). While the regulation of sirtuins by NAD^+ is likely, as we discuss in detail in the section titled Understanding the Functions of Lysine Acetylation through the Regulation of Sirtuins, there are many other ways

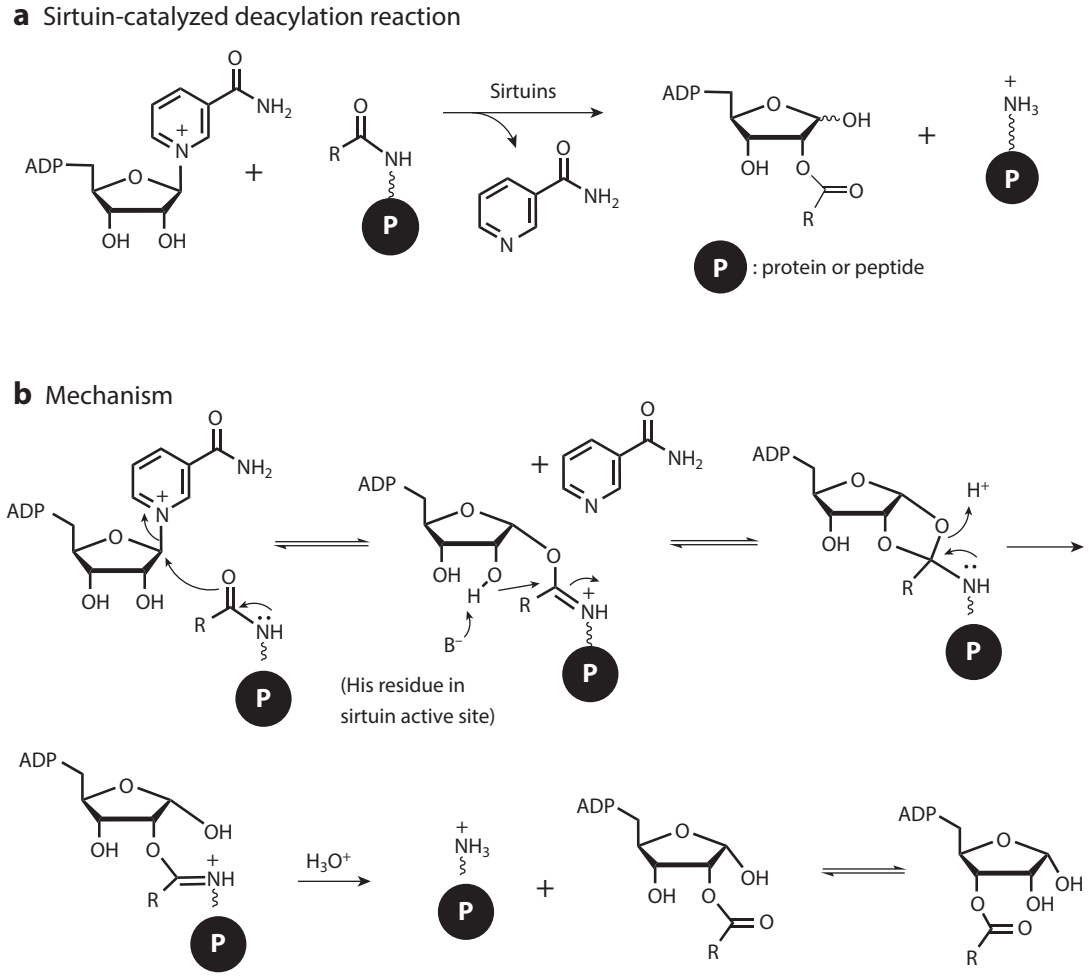

Figure 2

(*a*) The sirtuin-catalyzed deacylation reaction. (*b*) The reaction mechanism. For each deacylation reaction, one molecule of NAD^+ is consumed and nicotinamide and 2′-*O*-acyl ADP-ribose are produced.

to regulate the activities of sirtuins. It is the collective regulation by many different mechanisms that enables sirtuins to execute their various important biological functions.

The function of lysine acetylation is often achieved through its ability to mediate protein-protein interactions. In the epigenetic field, protein domains that bind to acetyl-lysine are called readers of acetyl-lysine. Bromodomains were the first such readers to be discovered (10). There are close to 50 proteins with bromodomains, some containing more than one (40). Most of them are involved in transcriptional regulation. YEATS domains and double PHD finger (DPF) domains have also been shown to be acetyl-lysine readers (41–44).

LYSINE ACETYLATION IN CHROMATIN REGULATION

Histone lysine acetylation is well known to be associated with active transcription. Several HATs are transcriptional coactivators, such as p300 and CBP. They are recruited by sequence-specific

transcription factors to promote the acetylation of nearby histones to promote transcription by RNA polymerase (RNA Pol) II. In contrast, many HDACs are known to suppress transcription. For example, SIRT6 associates specifically with the transcription factors HIF1α and NF-κB and deacetylates histones to suppress the transcription of target genes for these transcription factors. Similarly, SIRT7 is known to associate with Elk4 (45), NRF1 (46), and c-Myc (47) to repress target-gene expression by deacetylating histones. However, sometimes the deacetylases acting at chromatin can also promote transcription. This is particularly true for SIRT7, which is a well-known positive regulator of rDNA transcription by RNA Pol I (48) and is mainly localized to the nucleolus, where rDNA transcription and ribosome biogenesis occur.

The interesting question is, How does lysine acetylation promote transcription? Before explaining the different models that have been proposed, we note that transcription is a very complicated process, and therefore, any proposed model may not represent the whole picture. How lysine acetylation promotes transcription has been explained historically by two complementary models. First, by decreasing the positive charge of histone tails, lysine acetylation helps to change the chromatin structure, loosen the binding of histones to DNA, and thus, promote the unwinding of nucleosome DNA to promote transcription. Second, the acetyl-lysine is recognized by proteins containing specific domains that help promote transcription, such as bromodomains (40) and YEATS domains (41). These proteins include the bromodomain and extraterminal (BET) family of proteins, such as BRD4, which have been heavily explored pharmacologically for treating a variety of human cancers (49). It is thought that BRD4 binds to acetyl-lysines on histones and recruits other proteins, such as the mediator complex and pTEFb, which in turn recruit and activate RNA Pol II to transcribe the nearby genes.

In recent years, a new liquid-liquid phase separation or phase condensation model of transcription has been proposed (50), which is supported by imaging studies of different protein factors involved in transcription (51–56). In essence, the phase separation model says that transcriptional activation is achieved through the localized accumulation of various factors needed to recruit and activate RNA Pol II to synthesize specific mRNA. This localized accumulation, or phase condensation, is mediated by various noncovalent interactions among different biological molecules, including histones, sequence-specific transcription factors, coactivators, mediators, and RNA Pol II. In this context, the acetylation of lysine residues in histones and transcription factors and their recognition by specific domains provide a driving force for phase condensation. This model can explain many previous observations about transcription, including the ability of one enhancer to promote the transcription of multiple genes (50).

In support of the phase condensation model, multiple transcription factors and coactivators have been reported to form phase condensates during transcriptional activation (51–56). The well-known nucleolus is also a form of phase condensate that is responsible for ribosomal RNA transcription and processing. In the context of the phase separation model of transcription, it is also easy to understand why transcriptional coactivators are typically multidomain proteins. For example, p300 and CBP have approximately a dozen domains, including the HAT catalytic domain and a bromodomain that presumably binds to acetyl-lysine. The BRD4 coactivator also contains multiple domains, including two bromodomains. Most of these domains are important for mediating protein-protein interactions. Thus, a protein with multiple domains is well suited to form the multivalent interactions that are required for phase condensation.

The phase condensation model may also help explain the functional effects of lysine acetylation in DNA damage and repair. Many proteins are recruited to the site of DNA damage, just as many proteins are recruited during transcriptional activation. PTMs, such as acetylation, are likely to be important for mediating some of the protein-protein interactions required for phase condensation. Several HATs and HDACs are known to be important for DNA repair and are recruited to

DNA-damage sites. For example, two HATs, p300 and CBP, are recruited to double-strand break sites to facilitate SWI/SNF chromatin remodeling (57). SIRT6, which is important for both base excision repair and double-strand break repair (58, 59), is able to bind directly to DNA and is one of the earliest factors recruited to DNA-damage sites (60, 61). Likely, acetylation is needed for relaxing the chromatin and allowing recruitment of repair factors in the early phase of DNA damage repair, while deacetylation is needed in the later phase of DNA damage repair. Several studies support this model. SIRT1 has been reported to deacetylate and inhibit two acetyltransferases, TIP60 and MOF (62). Immediately following DNA damage, the binding of SIRT1 to TIP60 and MOF is transiently disrupted, leading to hyperacetylation and activation of TIP60 and MOF. Perhaps as the repair progresses, the interaction between SIRT1 and TI60/MOF resumes to bring things back to normal. Similarly, DBC1 (deleted in breast cancer 1), a known interacting protein and inhibitor of SIRT1, is reported to increase its binding to SIRT1 following DNA damage, presumably to transiently inhibit SIRT1 and allow acetylation to happen (63). In addition, several other reports have suggested that transient inhibition of SIRT1 activity or activation of acetyltransferase activity occurs following DNA damage (64–68). Another report also suggested that MOF acetylates DBC1 and inhibits its binding to SIRT1 (69). This is likely to form a negative feedback loop that allows the reactivation of SIRT1 as the repair process moves forward. In base excision repair, PCAF/GCN5-mediated acetylation of RPA1 is important for accumulating XPA at the DNA-damage site (70), while deacetylation of XPA by SIRT1 is also important (71), again suggesting that both acetylation and deacetylation are involved in DNA damage repair. p300 and CBP are recruited to DNA double-strand break sites and also regulate RAD52 acetylation, which is dynamically controlled by SIRT2 and SIRT3 (72). Several other proteins that are important in DNA damage sensing and repair are also regulated by reversible acetylation, including Ku70 (73), WRN (74), and NBS1 (75). Very likely, their acetylation is dynamically regulated according to the DNA damage repair status.

LYSINE ACETYLATION IN REGULATING PROTEIN STRUCTURE AND FUNCTION

Aside from chromatin biology and transcription, acetylation regulates many other proteins, including signaling proteins, structural proteins, and metabolic enzymes. Acetylation could regulate protein function in several different ways, which are summarized in this section (**Table 3**). Via these mechanisms, lysine acetylation could regulate numerous biological pathways. However, it is not necessarily the case that all lysine acetylation has an important function. This may be particularly true for mitochondrial-protein lysine acetylation, which may occur nonenzymatically (76). Although BLOC1S1 (biogenesis of lysosome-related organelles complex 1 subunit 1), which shares sequence similarity to GCN5, has been reported to be a mitochondrial acetyltransferase (77), more rigorous biochemical and structural studies are needed to fully establish the existence of mitochondrial HATs. If mitochondrial-protein lysine acetylation occurs nonenzymatically, it is possible that the mitochondrial deacetylase SIRT3 has evolved to remove acetyl-lysines that do have functional impact, while those without functional consequences are not touched.

Regulating Protein Interactions with Proteins, Nucleic Acids, or Small Molecules

Acetylation of lysine residues often affects a protein's interactions with other biological molecules. The exact molecular mechanism is often not known due to the lack of structural information, except for the binding of acetyl-lysine residues by reader proteins described above in the section

Table 3 Summary of nontranscriptional functions of lysine acetylation on protein structure and function

Function of acetylation	Specific function	Example of acetylation target	Deacetylase(s)
Regulating protein interactions	Disrupting protein-protein interaction	YAP2 (78), 14-3-3ζ (79), PPARγ (80)	SIRT1
		SKP2 (81, 82)	SIRT3, SIRT6
	Disrupting protein-nucleic acid interaction	MCM10	SIRT1
		PAF53, fibrillarin (84, 85)	SIRT7
	Disrupting protein-small molecule interaction	AKT (86)	SIRT1
Regulating protein stability	Destabilizing protein by promoting its binding to E3 ubiquitin ligases, leading to increased ubiquitination	PEPCK1 (87), BuBR1 (88, 89)	SIRT2
	Stabilizing protein by inhibiting ubiquitination	p53 (91–95)	SIRT1, SIRT6, SIRT7
		SMAD7 (96), CRTC2 (97), Notch (98)	SIRT1
		ACLY (99)	SIRT2
	Destabilizing target protein by promoting CMA	HSD17B4 (103)	SIRT3
		PKM2 (104)	SIRT2
	Stabilizing target protein by disrupting CMA	MST1 (105)	HDAC6
Regulating other posttranslational modifications	Competing with ubiquitination	p53 (91–95)	SIRT1, SIRT6, SIRT7
		SMAD7 (96), CRTC2 (97), Notch (98)	SIRT1
		ACLY (99)	SIRT2
	Competing with sumoylation	p300 (106)	SIRT1
	Promoting hydroxylation of the target protein	HIF1α (107)	SIRT2
	Suppressing phosphorylation of the target protein	NBS1 (75), S6K1 (110, 111)	SIRT1
		IRS2 (108, 109)	Unknown
	Promoting phosphorylation of the target protein	CREB (112)	SIRT1
		Keratin 8 (113)	SIRT2
Regulating enzymatic activity	Suppressing enzymatic activity by acetylating lysine residues that are important for catalysis	HMGCS2 (K310) (115), IDH2 (116), ACADL (117), ECHS1 (118)	SIRT3
		PGAM1 and PGAM2 (K100) (119, 120)	SIRT2
	Affecting enzymatic activities by acetylating allosteric lysine residues	G6PD (123)	SIRT2
		SHMT2 (124)	SIRT3
		HMGCS2 (K447) (115)	SIRT3
		PGAM1 (K253–254) (122)	SIRT1

Abbreviations: CMA, chaperone-mediated autophagy; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; IDH2, isocitrate dehydrogenase 2; PGAM, phosphoglycerate mutase; SHMT2, serine hydroxymethyltransferase 2.

titled Writers, Readers, and Erasers of Lysine Acetylation. Acetylation of YAP2, a protein important for regulating cell growth, negatively affects its interaction with TEAD4, and SIRT1 promotes the interaction by deacetylating YAP2 (78). Deacetylation of 14-3-3ζ by SIRT1 promotes its interaction with caspase 2 and inhibits apoptosis (79). PPARγ is deacetylated by SIRT1 at K268 and K293, which increases the binding of PPARγ to the brown adipose tissue program coactivator PRDM16, leading to a fat-browning effect of SIRT1 (80).

SKP2, an E3 ubiquitin ligase, is acetylated on two lysine residues that happen to be in the nuclear localization sequence. Thus, the acetylation of SKP2 disrupts its recognition by nuclear import proteins, leading to its cytosolic accumulation. SIRT3 and SIRT6 deacetylate SKP2 and promote its nuclear localization (81, 82). In this case, acetylation affects protein subcellular localization by regulating protein-protein interactions.

SIRT1 deacetylates MCM10 and promotes its interaction with DNA (83). SIRT7, which is known to promote rDNA transcription, works by deacetylating PAF53 and fibrillarin (an rRNA and histone H2A Gln104 methyltransferase) and promoting their binding to rDNA (84, 85).

Acetylation could also regulate protein binding to small molecules. For example, AKT is acetylated in the PH domain, and acetylation inhibits its binding to phosphatidylinositol triphosphate (PIP3). SIRT1, by deacetylating AKT, promotes its binding to PIP3 and activation (86).

Regulating Protein Stability by Changing Protein Degradation

Regulating protein stability is a very important means through which acetylation regulates protein function. First, acetylation can destabilize proteins. Acetylation promotes PEPCK1 degradation by increasing its interactions with UBR5, an E3 ubiquitin ligase (87). SIRT2 deacetylates PEPCK1 and stabilizes it to promote gluconeogenesis. Acetylation of the mitotic checkpoint kinase BuBR1 at K688 also promotes its proteasomal degradation, and SIRT2 deacetylates and stabilizes it (88, 89). In these cases, a likely explanation is that acetylation promotes binding by the E3 ubiquitin ligases, leading to increased ubiquitination and proteasomal degradation. ECHA, an enzyme involved in fatty acid oxidation, has recently been reported to be stabilized by SIRT3-mediated deacetylation. However, it is not clear how acetylation promotes ECHA degradation (90).

Acetylation can also stabilize certain proteins. An early example identified was the tumor suppressor p53. Acetylation of its C terminus by CBP/p300 stabilizes p53, while deacetylation by SIRT1, SIRT6, or SIRT7 promotes its degradation (91–95). SMAD7, a protein important for TGFβ signaling, is acetylated and stabilized by p300 but deacetylated and destabilized by SIRT1 (96). CREB-regulated transcriptional coactivator 2 (CRTC2), a protein that regulates hepatic gluconeogenesis, is stabilized by CBP/p300-catalyzed acetylation and destabilized by SIRT1-catalyzed deacetylation (97). Notch, which is important for intercellular signaling and tissue patterning, is similarly stabilized by acetylation and destabilized by SIRT1-catalyzed deacetylation (98). ACLY, a metabolic enzyme important for acetyl-CoA generation and fatty acid biosynthesis, is stabilized by PCAF-mediated acetylation and destabilized by SIRT2-mediated deacetylation (99). In these cases, the lysine residues that are acetylated are also ubiquitinated. Thus, acetylation promotes stability by blocking ubiquitination. There are also cases where acetylation stabilizes proteins, but it is not clear whether this occurs as a result of blocking ubiquitination or not. For example, the PER2 protein, which is important for the circadian clock, is stabilized by acetylation and destabilized by SIRT1 (100, 101), but the residues that are acetylated and ubiquitinated have not been determined, and therefore, the mechanistic detail is not clear.

Lysine acetylation could also affect protein stability by affecting a process called chaperone-mediated autophagy (CMA). CMA refers to the process that degrades selected cytosolic proteins in the lysosome with the help of heat-shock cognate 71-kDa protein (HSC70) (102). HSC70

recognizes a KFERQ motif on proteins and delivers them to lysosomes for degradation in a LAMP2A-dependent manner. The KFERQ motif requires a glutamine (Q), which can be flanked on either side by one or two positive residues (K or R), one or two hydrophobic residues (F, I, L, or V), and one negatively charged residue (E or D) (102). The exact order of the amino acids does not seem to matter (102). The sequence of this recognition motif, while not well defined and possibly difficult to identify, suggests that acetylation could regulate CMA-mediated degradation in two different ways: a KFERK_{Ac} motif could potentially mimic KFERQ to promote CMA, while a K_{Ac}FERQ motif might inhibit CMA. Both promotion and inhibition of CMA by acetylation have been reported in the literature. HSD17B4, an enzyme involved in fatty acid oxidation, is acetylated at K669 (sequence: KWTIDLK_{Ac}), and this acetylation promotes CMA. The acetylation and subsequent CMA-mediated degradation of HSD17B4 are regulated by CBP and SIRT3 (103). Acetylation of PKM2, a pyruvate kinase isoform that is often expressed in tumors, has also been reported to undergo CMA that is promoted by K305 acetylation (sequence: EK_{Ac}VFLAQK) (104). In contrast, for mammalian STE20-like kinase 1 (MST1), deacetylation of K35 (sequence: QPEEVFDVLEK_{Ac}) by HDAC6 promotes CMA (105).

Regulating Other Posttranslational Modifications

Just as the competition between acetylation and ubiquitination affects protein stability, acetylation could also compete with SUMO modification. The HAT protein p300 is acetylated on multiple lysine residues, including K1020 and K1024, which are both acetylated and sumoylated. SIRT1 thereby promotes p300 sumoylation by deacetylation (106).

Lysine acetylation also affects hydroxylation. HIF1α, a transcription factor that is important for the hypoxic response, is acetylated on K709 and deacetylated by SIRT2. Deacetylation promotes its binding to prolyl hydroxylase 2, a key regulator of HIF1α stability, and increases HIF1α hydroxylation and ubiquitination (107).

In addition, lysine acetylation affects phosphorylation. NBS1, a protein that is important for the DNA damage response, is deacetylated by SIRT1. This deacetylation is important for its S343 phosphorylation by ATM (75). Similarly, lysine acetylation of IRS2 inhibits its phosphorylation (108, 109). The phosphorylation of the mTOR substrate S6K1 at T389 is also promoted by SIRT1-mediated deacetylation (110, 111). Acetylation could also promote phosphorylation. CREB K136 acetylation promotes its cAMP-dependent phosphorylation, which in turn promotes glucogenic gene activation and suppresses hepatic lipid accumulation and secretion. SIRT1 overexpression deacetylates CREB K136, leading to suppressed gluconeogenesis but increased lipid accumulation (112). Keratin 8 K207 acetylation seems to promote S74 phosphorylation (113). In general, the mechanistic basis for how acetylation affects phosphorylation has not been investigated, but it is likely to occur through affecting interactions with the enzymes controlling phosphorylation.

Regulating Enzyme Activity by Affecting Catalytic or Allosteric Lysine Residues

Lysine residues could be situated within enzyme active sites and directly contribute to the binding of negatively charged substrates. In this case, acetylation would inhibit the enzymatic activity, and deacetylation would activate the enzyme. Examples of enzymes that are regulated in such a way include 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), which is reported to have three lysine residues that are deacetylated by SIRT3: K310, K447, and K473 (114). K310 is in the CoA-binding site, and therefore, its acetylation may negatively affect substrate binding (115). The other two acetylated lysine residues are not in the active site. K447 acetylation may affect the activity of

HMGCS2 by influencing oligomerization, since it is located in the oligomer interface (115). K473 acetylation may regulate the activity allosterically (114). Another example is isocitrate dehydrogenase 2 (IDH2), which is deacetylated and activated by SIRT3 on K413 (116). K413 is located in the $NADP^+$-binding site, and therefore, its acetylation is likely to decrease $NADP^+$ binding. Long-chain acyl-CoA dehydrogenase (ACADL) is reported to be acetylated on K318 and K322, which are located near the FAD-binding site (117). Deacetylation of these two residues by SIRT3 promotes ACADL activity (117). Enoyl-CoA hydratase-1 (ECHS1) is also regulated by acetylation on K101, which is located in the CoA-binding site. This acetylation is reversed by SIRT3 to promote its activity (118). Phosphoglycerate mutase (PGAM)1 and PGAM2 are acetylated at K100 (119, 120). K100 is directly involved in substrate binding, based on an available structure (121), and not surprisingly, deacetylation by SIRT2 promotes activity. Interestingly, PGAM1 is also regulated by acetylation at the very C-terminal residues K253–254, and deacetylation at this site by SIRT1 inhibits its activity. This region is known to cap the active site (122). While the exact mechanism by which acetylation of K253–254 promotes PGAM1 activity is not clear, we speculate that when these residues are deacetylated, they may compete with K100 for binding to the substrate, which leads to an unproductive substrate-enzyme binding conformation that inhibits the enzyme.

Acetylation can also affect enzyme oligomerization. Almost all mammalian metabolic enzymes form functional oligomers. Thus, affecting oligomerization could readily impact their enzymatic activity. Glucose 6-phosphate dehydrogenase (G6PD), an enzyme in the pentose phosphate pathway that is important for NADPH production and nucleotide biosynthesis, is active as a dimer and tetramer. Acetylation of K403, located in the dimerization interface, disrupts dimerization. SIRT2 catalyzes its deacetylation to activate G6PD activity and promote NADPH production (123). Serine hydroxymethyltransferase 2 (SHMT2), which exists as a functional tetramer, is reported to be acetylated on K95, which is located in the oligomer interface. Acetylation or mutation of this residue to either Gln or Arg negatively impacted the enzyme's tetramerization and activity (124). SIRT3 deacetylates SHMT2 to promote its activity and thus cell growth (124).

There are also cases where lysine acetylation affects enzymatic activity, but the exact mechanism is not obvious. This is the case for some of the first metabolic enzymes that were identified as being regulated by acetylation. Acetyl-CoA synthetase 1 and 2 (encoded by the genes *ACSS2* and *ACSS1*, respectively) are deacetylated and activated by SIRT1 and SIRT3, respectively (125, 126). In *Salmonella*, acetyl-CoA synthetase is similarly regulated by lysine acetylation (127). In the available *Salmonella* acetyl-CoA synthetase structure, the acetylated lysine residue is not in the active site, but the authors suggest that this enzyme catalyzes two half-reactions; the lysine residue is important for the first step, while the structure captures a different conformation that is required for the second step (127, 128).

UNDERSTANDING THE FUNCTIONS OF LYSINE ACETYLATION THROUGH THE REGULATION OF SIRTUINS

Lysine acetylation is often compared to phosphorylation and ubiquitination because of the large number of proteins that are known to be modified and regulated by acetylation. Proteomic studies have identified ~2,000 proteins that contain lysine acetylation (4, 5). Research over the past 20 years has led to the identification of over 100 substrate proteins for SIRT1 [for substrate tables, see two recent reviews (129, 130)]. The number of confirmed substrates for other sirtuins, even though relatively small, is growing. As more deacetylation substrates are identified for sirtuins, more opportunities become available to understand the function of lysine acetylation. However, at the same time, the identification of additional substrates could also raise additional questions:

Why does a particular sirtuin enzyme work with so many different substrates? What is the logic for using a particular sirtuin to deacetylate a particular substrate protein?

From what we know about protein phosphorylation, PTMs are well suited to mediating cell signaling because of the ability of protein kinases to respond to various signals and stresses. For example, receptor tyrosine kinases are turned on when they are bound by an extracellular ligand. Similarly, mTOR is activated by amino acids and growth factor signaling, and AMPK is activated by AMP. In other words, it is the regulatory properties of the protein kinases that make them suitable for sensing and responding to signals and stresses. Therefore, to fully understand the functions of an enzyme that regulates PTMs, it is essential to understand how the enzyme itself is regulated. Imagine how little we would appreciate the function of AMPK if we did not know that it is activated by AMP. The same principle applies to the protein lysine deacetylases, the sirtuins. Therefore, it is important to consider how the sirtuins are regulated if we wish to understand the logic behind using acetylation.

The Multiple Regulatory Mechanisms of SIRT1

SIRT1 is the most studied mammalian sirtuin, and its regulation is also the best understood (131). It is regulated by noncovalent interactions with metabolites or proteins, PTMs, degradation, and transcriptional control. Shortly after the discovery that sirtuins use NAD^+ as their cosubstrate to deacetylate lysine residues, it was proposed that sirtuins may be regulated by NAD^+ levels (99). For example, when the glucose levels are high, NADH levels are also high, while NAD^+ levels are low (because glycolysis converts NAD^+ to NADH), and sirtuins may be less active. In contrast, under low-glucose conditions (caloric restriction), NAD^+ levels are high, and sirtuins are more active (132). This may be the reason why sirtuins are important for the effects of caloric restriction on longevity that are observed in many model organisms. This line of thinking has had a great impact in the sirtuin field, as it boosted studies that help to understand the interplay between metabolism and sirtuins. A lot of researchers were also excited to explore the health benefits of NAD^+-boosting small molecules (39). However, experimentally proving this hypothesis is difficult, and several misconceptions are worth clarifying here. First, it is unlikely that NADH inhibits sirtuin in cells, because physiological NADH concentrations are orders of magnitude lower than those of NAD^+, and NADH is not a good inhibitor of sirtuins (133–135). Second, if we believe that the NAD^+ concentration is orders of magnitude higher than the NADH concentration in cells, then caloric restriction may not lead to a significant change in NAD^+ concentration. Therefore, the regulation of sirtuin activity by caloric restriction via NAD^+ may involve other pathways such as NAD biosynthesis or additional regulatory mechanisms that are discussed in the next few paragraphs (134). We believe that although the NAD^+ concentration contributes to the regulation of some sirtuins, this is only a minor mechanism for the regulation of sirtuins, as many other regulatory mechanisms could serve similar purposes (**Figure 3**).

SIRT1 is inhibited by ATP binding to the C-terminal domain (136). ATP binding leads to a less compact structure and affects the association of the C-terminal domain with the catalytic domain, as well as substrate recruitment. This could be another mechanism that contributes to SIRT1 activation during caloric restriction. Another endogenous metabolite, *N*-methyl nicotinamide, is reported to stabilize SIRT1 protein, but the exact molecular mechanism is not entirely clear (137).

SIRT1 can be phosphorylated by several kinases, and these modifications regulate its activity and contribute to its role in regulating DNA damage responses. SIRT1 is phosphorylated at four serine residues (S154, 649, 651, and 683 in murine SIRT1) by CK2 after exposure to ionizing radiation. These phosphorylation events activate SIRT1's deacetylase activity (138). This mechanism of regulation is consistent with the reported positive roles of SIRT1 in DNA damage and

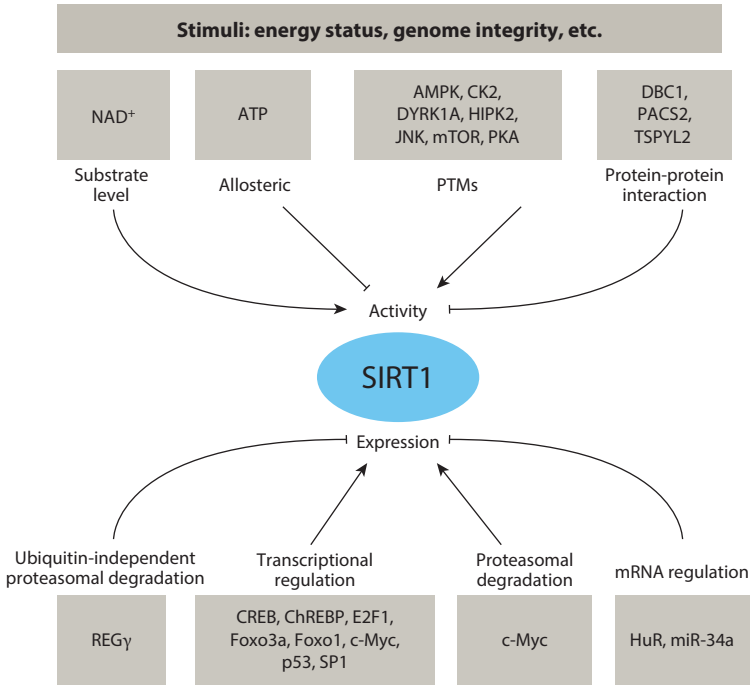

Figure 3

Various regulatory mechanisms for SIRT1 that contribute to its biological functions, namely transcription, DNA damage repair, and metabolism. Mechanisms that regulate the activity of SIRT1 are shown at the top and those that regulate the level of SIRT1 are shown at the bottom. These regulatory mechanisms allow SIRT1 to sense various stimuli, including energy status and DNA damage. The regulation by kinases or transcription factors could either promote or inhibit SIRT1, depending on the kinases or transcription factors. However, to simplify the figure, a single arrow is used to indicate the regulation. Abbreviations: DBC1, deleted in breast cancer 1; DYRK1A, dual specificity tyrosine phosphorylation-regulated kinase 1A; PACS2, phosphofurin acidic cluster sorting protein 2; PTM, posttranslational modification.

repair. A different report shows that CK2 can phosphorylate S164 in obese mice, which inhibits SIRT1 activity (139). After severe DNA damage, SIRT1 is phosphorylated by HIPK2 at S682 and inhibited. This inhibition facilitates the activation of p53 to induce cell death (67). Dual specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) and related DYRK3 phosphorylate T522 of murine SIRT1 (corresponding to T530 of human SIRT1) and activate SIRT1 (140). Activated SIRT1 inhibits p53, which protects the cells from death. JNK1/2 phosphorylate SIRT1 at S27, S47, and T530 (141, 142). This JNK-mediated phosphorylation promotes the deacetylation of H3 but not p53 (142). JNK-mediated activation of SIRT1 may explain the beneficial effects of SIRT1 under certain stresses, as JNKs are activated under stressful conditions such as oxidative stress (143). However, prolonged JNK1 (but not JNK2) activation in obese mice leads to degradation of SIRT1 in the liver, hinting at a more complex picture of its regulation by JNK (144).

Regulation by phosphorylation also contributes to the role of SIRT1 in metabolism. Phosphorylation of SIRT1 on S47 by mTOR inhibits SIRT1 (145). As mTOR is a nutrient sensor, this regulation could potentially contribute to the regulation of SIRT1 activity by the metabolic state. cAMP/PKA phosphorylates SIRT1 on S434 in the catalytic domain to activate SIRT1. This regulation allows the adrenergic signaling cascade to activate SIRT1 to control metabolic responses via

activation of PGC-1α. During cold stress, adrenergic stimulation increases fatty acid consumption and energy metabolism to increase heat production in brown adipose tissue (146). Some of the well-studied biological functions of SIRT1 are to promote lipolysis and mitochondrial metabolism through PGC-1α (147, 148) and to inhibit lipogenesis by inhibiting PPARγ (149) and SREBP-1 (150, 151). Therefore, the activation of SIRT1 by cAMP/PKA during conditions of higher energy expenditure would be beneficial.

SIRT1 has been reported to be regulated by ubiquitin-independent proteasomal degradation via REGγ, the 11S proteasome regulatory complex (152). REGγ promotes the degradation of SIRT1. In REGγ-knockout mice, SIRT1 is upregulated in the liver to promote autophagy-mediated lipid metabolism, leading to decreased liver steatosis. This study also revealed that the REGγ and SIRT1 interaction is further regulated by the energy status of cells. In response to glucose depletion, AMPK phosphorylates T530 of SIRT1, leading to the dissociation of SIRT1 from REGγ and the activation of SIRT1. Thus, the mechanisms regulating SIRT1 via AMPK-mediated phosphorylation and REGγ-mediated proteasomal degradation are intimately connected (152). Interestingly, REGγ is also reported to be inhibited by SIRT1-mediated deacetylation at K195 (153). Regulation could thus be bidirectional and constitute a feedback loop; i.e., increased SIRT1 due to lack of REGγ activity leads to SIRT1-mediated deacetylation, which further inhibits REGγ.

SIRT1 is also regulated by protein-protein interactions. One of the first mechanisms of regulation of SIRT1 to be identified was inhibition by DBC1 (154, 155). DBC1 interacts with and inhibits SIRT1, leading to increased p53 acetylation and apoptotic activity. Regulation by DBC1-SIRT1 interaction is further influenced by dietary conditions, DNA damage, and other signaling pathways. A high-fat diet promotes the DBC1-SIRT1 interaction and contributes to the inhibition of SIRT1. DBC1 deletion protects mice from high-fat diet–induced liver steatosis and inflammation (156). After DNA damage, ATM phosphorylates DBC1 at T454 to increase its interaction with SIRT1 (63, 64). DBC1 phosphorylation by ATM/ATR also promotes its sumoylation, which in turn promotes its interaction with SIRT1 in response to DNA damage (66). Another DNA damage–induced kinase, Chk2, is reported to phosphorylate REGγ on S247, which promotes REGγ-DBC1 interaction with SIRT1 and causes SIRT1 inhibition. Interestingly, SIRT1 also deacetylates Chk2 on K235 and K249 and inhibits Chk2 (157). Thus, SIRT1 and Chk2 negatively regulate each other. AMPK and PKA activation also lead to dissociation of DBC1 from SIRT1 and activation of SIRT1 (158). The effect of AMPK seems to be mediated through SIRT1 T344 phosphorylation (159). Interestingly, the interaction between DBC1 and SIRT1 is also regulated by acetylation (69, 160). DBC1 K112 and K215 are acetylated by MOF and deacetylated by SIRT1. Acetylation inhibits binding to SIRT1, while deacetylation promotes it. Thus, by deacetylating DBC1, SIRT1 could negatively regulate its own activity, forming a negative feedback loop (69).

Another negative regulator of SIRT1 is TSPYL2. DNA damage promotes the binding of TSPYL2 to SIRT1, leading to SIRT1 inhibition and increased p300 acetylation and activity. This in turn promotes p53 stabilization and activity (68). Cells lacking TSPYL2 are unable to activate p53 after DNA damage due to increased deacetylation and destabilization of p53 by SIRT1. Interestingly, TSPYL2 is upregulated in DBC1-deficient cells. Therefore, it appears that TSPYL2 is brought in to inhibit SIRT1 when DBC1 is not present (68). However, TSPYL2 differs from DBC1 in that it also binds and promotes p300 activity (68).

Phosphofurin acidic cluster sorting protein 2 (PACS2) has also been reported to be a negative regulator of SIRT1 (161). Interestingly, PACS2 cooperates with DBC1, and the two together form a tight complex with SIRT1. Insulin signaling activates AKT, which phosphorylates PACS2 S437 and promotes its interaction with DBC1 and SIRT1 (162). This provides another mechanism for the inhibition of SIRT1 activity under conditions of glucose abundance.

SIRT1 is also regulated at the transcriptional level. Consistent with SIRT1's role in caloric restriction, Foxo3a and p53 together promote the transcription of SIRT1 under starvation conditions. The SIRT1 promoter contains two p53-binding sites, and starvation-induced SIRT1 expression requires p53 (163). Given that SIRT1 inhibits p53 and Foxo3a, the activation of SIRT1 expression by p53 and Foxo3a constitutes a negative feedback mechanism. Similarly, under caloric restriction, CREB has been reported to activate SIRT1 transcription, whereas under feeding conditions, ChREBP suppresses SIRT1 transcription (164). In the brain, CREB promotes SIRT1 expression, which in turn promotes CREB transcriptional activity (165). A similar regulatory loop also exists for FOXO1 and SIRT1, with FOXO1 promoting SIRT1 transcription, and SIRT1 activating FOXO1 activity by deacetylation (166). PPARβ/δ has been shown to promote SIRT1 transcription indirectly via Sp1, which binds the SIRT1 promoter (167). In contrast, PPARγ, which is important for adipogenesis, binds to the SIRT1 promoter and represses its transcription (168). E2F1, which is activated by DNA damage, promotes SIRT1 transcription to suppress cell death, and SIRT1 deacetylates E2F1 to decrease its transcriptional activity, forming a negative feedback loop (169).

Certain proteins have been reported to regulate SIRT1 via multiple mechanisms. c-Myc was shown to upregulate SIRT1 transcription in a p53-dependent manner due to competing binding of p53 and c-Myc to the SIRT1 promoter (170). c-Myc induction increases SIRT1 protein levels by inhibiting proteasomal degradation, but the exact mechanism is not clear (171). c-Myc further enhances SIRT1 activity by binding to DBC1 and by increasing NAD^+ biosynthesis. Activated SIRT1 in turn helps to limit c-Myc-induced apoptosis, which occurs partially through regulating p53 (171). SIRT1 also deacetylates c-Myc and increases its interaction with Max, a partner that is important for c-Myc function (172).

In addition to the aforementioned regulatory mechanisms, SIRT1 is further regulated by the RNA-binding protein HuR (173), various microRNAs (miRNAs), and long noncoding RNAs (lncRNAs) (for a recent review of the miRNAs and lncRNAs that regulate SIRT1, see 130). One interesting case of SIRT1 regulation by miRNA is that by miR-34a. miR-34a inhibits SIRT1 expression, which leads to p53 stabilization and activation. miR-34a itself is a target of p53 transcriptional activity. Thus, p53 promotes its own activity via miR-34a suppression of SIRT1, forming a positive feedback loop (174).

It is almost daunting to know that SIRT1 is regulated by so many different mechanisms. However, in many cases, we start to understand the function of SIRT1 through these regulatory mechanisms. SIRT1 regulates certain pathways by deacetylating a set of substrate proteins. If regulation of these pathways by SIRT1 helps cells and/or organisms to achieve homeostasis or activate specific functions under a particular condition, then the regulatory mechanisms are used to elevate SIRT1 activity or expression under that condition. In contrast, if regulation of these pathways by SIRT1 negatively impacts the cellular function under a particular condition, then SIRT1 tends to be downregulated. The ability to tune SIRT1 activity in response to different cues enables acetylation to have a signaling function, just as the ability to tune kinase activity allows phosphorylation to mediate cell signaling. The ability to control these PTMs by regulating the PTM enzymes enables cells and organisms to respond to stresses and signals to achieve homeostasis and proper function.

Regulation of SIRT2

SIRT2 deacetylates over three dozen substrate proteins and regulates a variety of biological processes, including suppressing glycolysis (119, 120, 175) and lipogenesis (99, 176, 177); promoting gluconeogenesis (87), glucose uptake (178), and the pentose phosphate pathway (123); relieving

oxidative stress (179–181) but promoting oxidative stress-induced cell death (182); promoting cell cycling and division (88, 89, 183–188); promoting bacterial pathogenesis (189–192); suppressing or promoting inflammation (193–203); protecting or exacerbating neurodegeneration (204–211); and either suppressing or promoting tumorigenesis, depending on the context (183, 212, 213). The sometimes contradictory roles of SIRT2 suggest that much remains to be understood about its regulation and how that regulation is connected to its biological functions. As an example, SIRT2 has been reported to act as a tumor suppressor, because SIRT2 knockout mice develop more tumors as they age (183, 184); however, other reports show that knockdown and pharmacological inhibition of SIRT2 suppress cancer in cell culture and mouse models (212–215). We think that these seemingly conflicting reports could actually be explained from a functional and regulatory perspective. On the one hand, SIRT2 is a stress-response protein, and therefore, there are elevated stresses in SIRT2 knockout mice, such as oxidative stress, which can cause DNA damage and thus promote tumor development. On the other hand, cancer cells are likely to be more dependent on SIRT2 to deal with elevated stresses. Therefore, inhibiting SIRT2 leads to overwhelming stresses in cancer cells and, thus, cancer cell death. In other words, SIRT2 could represent a nononcogene addiction of cancer cells. From this perspective, it is important to identify the exact stress responses that are dependent on SIRT2 and can upregulate the expression and/or activity of SIRT2.

SIRT2 is downregulated in adipogenesis and upregulated by caloric restriction in adipose tissue (176, 179). In dietary obesity–associated HIF1α activation, HIF1α directly suppresses the transcription of SIRT2, leading to the restriction of fatty acid oxidation and energy expenditure (216). This activity is consistent with the role of SIRT2 in suppressing lipogenesis. Interestingly, SIRT2 is also known to deacetylate HIF1α and promote its degradation (107). Thus, SIRT2 and HIF1α negatively regulate each other. Seryl-tRNA synthetase has also been reported to recruit SIRT2 to chromatin to suppress the transcription of the VEGFA gene and thus inhibit vascularization (217). This is consistent with the negative cross regulation of SIRT2 and HIF1α, as HIF1α is well known to promote vascularization. SIRT2 mRNA is targeted by several miRNAs (175, 218–221). One interesting finding is that miR200-C downregulates SIRT2 to promote a Warburg-like effect in primed human pluripotent stem cells (175). This is also consistent with the negative regulation of HIF1α by SIRT2, as HIF1α is an important transcription factor that promotes the Warburg effect. Oxidative stress is reported to activate SIRT2 expression (179, 222), which is consistent with the role of SIRT2 in promoting NADPH production (123).

Multiple regulatory mechanisms connect to the role of SIRT2 in the cell cycle. During the G2/M transition, SIRT2 is phosphorylated at multiple sites, and this increases its stability (223). C-terminal S368 phosphorylation, controlled by CDK1 and CDC14A/B, seems to affect SIRT2's role in the cell cycle (224). S331 is phosphorylated by CDK2 and CKD5, and this phosphorylation is important for the role of SIRT2 in suppressing neurite outgrowth (225). A flavin protein, NQO1, also binds and activates SIRT2 to promote mitosis (226).

Regulation of SIRT3

SIRT3 is mainly localized to the mitochondria, where it promotes mitochondrial metabolism and suppresses oxidative stress by deacetylating and activating many mitochondrial enzymes. Consistent with its role in suppressing oxidative stress, SIRT3 is transcriptionally activated by the oxidative stress sensor NRF2 (227). SIRT3 also promotes oxidative phosphorylation and suppresses the Warburg effect (228). These activities could manifest differently in cancer phenotypes. By suppressing the Warburg effect, SIRT3 inhibits cancer cell growth (2D growth or attached growth) (228). However, because of its ability to reduce oxidative stress, SIRT3 promotes anchorage-independent growth of cancer cells by enabling the oxidative stress caused by detachment from the extracellular matrix to be dealt with (229). Accordingly, SIRT3 is downregulated

during attached growth but upregulated during cell detachment (230). SIRT3 is transcriptionally activated by a complex formed between a splicing variant of metadherin and NF-κB p65 to promote epithelial-to-mesenchymal transition (231). Fasting has long been reported to increase SIRT3 levels in the liver and brown adipose tissue, but the mechanism is still not well understood (232, 233). It is likely through cAMP/PKA/CREB signaling, as cAMP has been reported to increase SIRT3 expression (234), similar to the regulation of SIRT1 by CREB (165).

Another very interesting mechanism of SIRT3 regulation involves protein-protein interactions. Using a proteomic strategy, SIRT3 was shown to interact with many mitochondrial proteins. In healthy mitochondria, SIRT3 prefers to interact with ATP synthase at the inner mitochondrial membrane. This interaction is pH dependent due to H135 protonation of ATP5O subunits. Upon matrix pH reduction and mitochondrial depolarization, SIRT3 dissociates from ATP synthase to interact with and deacetylate many other mitochondrial proteins. In this way, SIRT3 activates many mitochondrial proteins to help restore depolarized mitochondria (235). This regulation is consistent with the known effect of SIRT3 in promoting mitochondrial metabolism and allows SIRT3 to sense mitochondrial health status.

Regulation of SIRT6

Many reported mechanisms of regulation of SIRT6 can also be connected to its known biological functions. Consistent with the role of SIRT6 in DNA damage repair, ATM phosphorylates SIRT6 at S112, which increases its stability by inhibiting MDM2-mediated ubiquitination at K346 (236). JNK phosphorylates S10 of SIRT6 to promote nonhomologous end joining (237).

SIRT6 suppresses the function of several transcription factors and signaling pathways, and many of these transcription factors and signaling pathways also regulate SIRT6, forming feedback loops. The transcription factor p53 promotes the expression of miR-34a, which inhibits SIRT6 (238). As SIRT6 is reported to deacetylate and destabilize p53 (93), the regulation of SIRT6 by miR-34a may form a positive feedback loop for p53 function. SIRT6 is known to suppress SMAD2 (239, 240) and SMAD3 (241–243), and TGFβ signaling has been reported to increase SIRT6 expression, suggesting that SIRT6 may be part of a negative feedback loop for SMAD2/3 regulation. SIRT6 is well known to suppress AKT signaling (244, 245). Accordingly, AKT1 could phosphorylate S338 of SIRT6 to promote its interaction with MDM2 and subsequent degradation (246). Furthermore, AKT could suppress Foxo3a to suppress SIRT6 transcription (247, 248).

SIRT6 has also been reported to be regulated by free long-chain fatty acids, such as palmitic acid, in vitro (249). SIRT6 is able to remove both acetyl and long-chain fatty acyl groups (250, 251). Interestingly, palmitic acid promotes SIRT6's removal of acetyl groups but modestly inhibits its ability to remove long-chain fatty acyl groups (249, 251). The concentration of long-chain fatty acids required to activate SIRT6 is on the order of 100 μM (249), which is higher than the physiological concentration of free fatty acids, and therefore, the physiological significance of this regulatory mechanism needs to be further investigated. However, the possibility that lipid molecules might regulate SIRT6 activity is extremely interesting and has motivated the development of synthetic small molecule activators of SIRT6 (252–254).

Regulation of SIRT7

Several reported mechanisms regulating SIRT7 are also connected to its known biological functions. One of the earliest functions discovered for SIRT7 was its ability to promote rDNA transcription and ribosome biogenesis (48, 255) by deacetylating several proteins involved in rDNA

transcription and splicing (84, 256, 257). Low-glucose conditions, AMPK activation, or inhibition of transcription lead to the release of SIRT7 from nucleoli (84), where rDNA transcription and biogenesis occur, and promote the degradation of SIRT7 (258). This makes sense, as under these conditions, downregulation of rRNA synthesis would be beneficial. SIRT7 is also known to be recruited by several transcription factors (Elk4, c-Myc, and NRF1) to deacetylate histone H3K18 and suppress Pol II–mediated transcription (45–47). Interestingly, under low-energy conditions, AMPK activation could inhibit the methylation of SIRT7 by PRMT6, promoting its H3K18 deacetylation activity (259). Thus, the inhibition of SIRT7's effects on Pol I– and Pol II–mediated transcription may be differentially regulated to coordinate the response to cellular stress. The c-Myc connection is of particular interest, as c-Myc is known to promote the transcription of ribosomal proteins. Therefore, the regulation of SIRT7 via these mechanisms is positioned to coordinate the synthesis of rRNA and ribosomal proteins in response to cellular stress.

Specificity and Cross Talk Among Different Sirtuin Regulatory Mechanisms

We discuss many regulatory mechanisms of sirtuins because they provide important insights into the functions of these proteins. Interesting questions that arise include whether different regulatory mechanisms are pathway specific and whether there is cross talk among the mechanisms regulating different pathways. Although regulatory mechanisms that change sirtuin transcription or protein levels may affect all pathways, posttranslational mechanisms in principle have the ability to be pathway specific, as a PTM may affect only the binding of specific substrate proteins. For example, JNK-mediated phosphorylation of SIRT1 promotes the deacetylation of H3 but not p53 (123). This, in turn, could allow cross talk between different pathways. One example is the regulation of SIRT7 by AMPK. SIRT7 has two major known functions: promoting rRNA synthesis and inhibiting transcription factors such as c-Myc, which promotes the transcription of genes encoding ribosomal proteins. AMPK inhibits SIRT7's effects on rRNA synthesis (258) but promotes its suppression of c-Myc (259). Under low-energy conditions, cells would presumably want to limit the transcription of both rRNA and genes encoding ribosomal proteins via this regulatory mechanism.

OTHER ACYL-LYSINE MODIFICATIONS

Cells contain various other acyl-CoA species in addition to acetyl-CoA. Over the past decade, these acyl-CoA molecules have been shown to also modify protein lysine residues, leading to various acyl-lysine modifications, including protein lysine butyrylation (Kbu) and propionylation (Kpr) (260), lysine crotonylation (Kcr) (261), lysine 2-hydroxyisobutyrylation (Khib), and lysine β-hydroxybutyrylation (Kbhb) (262, 263). As for lysine long-chain fatty acylation (KFA), although it was initially reported in the 1990s (264, 265), its biological function was not established until recently (23). Information about the writers, erasers, and readers of these acyl-lysine marks is summarized in **Tables 1** and **2**.

Malonylation, Succinylation, and Glutarylation

Lysine malonylation (Kmal), succinylation (Ksuc), and glutarylation (Kglu) are negatively charged lysine acylations that were initially reported in 2011 and 2014 (22, 266). At physiological pH, Kmal, Ksuc, and Kglu reverse the charge status of the unmodified lysine residue from positive (+1) to negative (−1), a more substantial change than is observed with other lysine acylations. Ksuc and

Kmal are abundant and found on hundreds of proteins in budding yeast, bacteria, and mammals (22, 266–276). Ksuc is more abundant on mitochondrial proteins while Kmal is more abundant on cytosolic proteins.

Ksuc was discovered through both mass spectrometry– (277) and enzymology-based studies (22) at approximately the same time. In a crystal structure of SIRT5 in complex with thioacetyl-lysine peptide, a buffer molecule interacts with the Tyr102 and Arg105 residues in the catalytic pocket of SIRT5 through its negatively charged sulfonate group. Moreover, the sulfonate group is also close to the thioacetyl-lysine group (22). These observations lead to the hypothesis that SIRT5 binds more strongly and is more reactive toward a negatively charged acyl-lysine moiety, such as Kmal or Ksuc (22). Indeed, this hypothesis has been proven true using in vitro enzymology studies (22). CobB, a known Sir2-like bacterial lysine deacylase, has desuccinylase activity, suggesting that the regulatory mechanism is evolutionarily conserved (278). The SIRT5 study is interesting as it not only leads to the identification of new acyl-lysine modifications but also identifies the major eraser of these modifications. In addition to SIRT5, SIRT7 is reported to desuccinylate histone H3K122 and deglutarylate histone H4K91 (279, 280). Several other negatively charged acyl-lysine modifications resulting from a few acyl-CoA molecules in branched-chain amino acid metabolism have also been reported. These modifications can be reversed by SIRT4, but the functions of these modifications have not been extensively investigated (281).

KAT2A, a known acetyltransferase, is reported to be the Ksuc writer on histone H3K79 and Kglu writer on H4K91 (280, 282). Interestingly, to compensate for the low concentration of succinyl-CoA in the nucleus, KAT2A interacts with the α-ketoglutarate dehydrogenase (α-KGDH) complex in the nucleus to access succinyl-CoA generated locally by the α-KGDH complex on chromatin (282).

Despite the report that KAT2A is a Ksuc and Kglu writer, the consensus in the field is that most Ksuc and Kglu events in the mitochondria occur nonenzymatically. This is because succinyl-CoA and glutaryl-CoA can chemically acylate proteins on lysine residues rather efficiently. In contrast, other acyl-CoA molecules, such as acetyl-CoA and butyryl-CoA, have a limited ability to chemically acylate proteins (283). The high chemical reactivity of succinyl-CoA and glutaryl-CoA can be explained by intramolecular general base catalysis, where the terminal carboxyl group attacks the acyl-carbonyl carbon, leading to the production of a highly reactive five- or six-membered anhydride ring intermediate (283). Furthermore, since anhydride is also reactive toward lysine residues at acidic pH, such nonenzymatic acylation may also occur in organelles other than mitochondria.

Ksuc and Kglu predominately occur on mitochondrial metabolic enzymes, and therefore, Ksuc and Kglu play important roles in regulating mitochondrial functions such as respiration, fatty acid oxidation, and ketogenesis. Mechanistically, the negatively charged acylations regulate the enzymatic activity or stability of proteins, as summarized below.

Consistent with the nonenzymatic acylation, negatively charged acylations often inhibit the activity of substrate proteins. These include HMGCS2, the rate-limiting ketogenic enzyme (270); IDH2; mitochondrial trifunctional enzyme subunit A (ECHA), the enzyme involved in fatty acid β-oxidation (271); carbamoyl phosphate synthase 1 (22, 266); uncoupling protein 1 (276); glutaminase (GLS) (284); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and PKM2, two nonmitochondrial substrates involved in glycolysis (275, 285). In several cases, the negatively charged Ksuc disrupts the binding of substrates to the enzymes (268, 270, 286). However, in the case of GLS, Ksuc could also regulate protein stability, as the Ksuc of GLS K164 promotes subsequent ubiquitination at residue K158 (284). However, more examples are needed to fully establish Ksuc as a regulator of protein stability and to understand its mechanism of action.

So far, only two proteins have been reported to be activated by Ksuc: pyruvate dehydrogenase complex and succinate dehydrogenase A (269, 287). However, another report suggested that

hypersuccinylation could inhibit their activity (288). In the latter case, hypersuccinylation is caused by IDH1 mutants that produce the oncometabolite 2-hydroxyglutarate, which inhibits succinate dehydrogenase and promotes succinyl-CoA accumulation.

A few studies have implicated the role of negatively charged acyl-lysine modifications in epigenetics and cross talk between metabolism and epigenetics. Histone Ksuc generally correlates with active gene expression (282, 289), which is perturbed by defects in the tricarboxylic acid cycle (290). SIRT7 has been reported to remove Ksuc on histone H3K122 and thereby promote chromatin condensation and DNA double-strand break repair (279). Since SIRT7 does not have the conserved Arg105 residue that allows SIRT5 to accommodate negatively charged acylation, in-depth enzymology studies are needed to understand this activity.

Ksuc inhibits PKM2 by promoting its tetramer to dimer transition (275). Interestingly, Ksuc-induced PKM2 dimers accumulate in the nucleus, where the PKM2-HIF1α complex is formed at the promoter of the IL-1β gene in macrophages to promote an inflammatory response (275). This study therefore revealed a novel role of SIRT5 in inhibiting inflammation and preventing dextran sulfate sodium–induced colitis in mice (275).

Long-Chain Fatty Acylation

KFA was discovered in the early 1990s (264, 265, 291). KFAs have distinct structural and biochemical properties because they are highly hydrophobic and often regulate the membrane binding and protein-protein interactions of their substrate proteins (292).

Several sirtuins and HDACs, including SIRT6, SIRT2, and HDAC11, can remove long-chain fatty acyl groups, and their physiological substrates have been identified. Other HDACs and sirtuins (SIRT1, SIRT3, SIRT7, and HDAC8) have also been reported to have this activity in vitro, but their physiological substrates have not been reported (18, 24, 249, 293). SIRT6 exerts much stronger (by 300-fold) catalytic activities in vitro toward long-chain fatty acyl peptides than acetyl peptides (23). Crystal structure analysis suggested that SIRT6 has a unique, large hydrophobic pocket that enables it to accommodate myristoyl-lysine (23).

TNF-α was the first mammalian lysine long-chain fatty acylated substrates to be discovered, in 1992 (264). TNF-α is a proinflammatory cytokine that is primarily produced as a type II transmembrane protein (mTNF-α). At the plasma membrane, mTNF-α is cleaved to release the C-terminal domain, namely secreted TNF-α (sTNF-α). The acylated lysine residues K19 and K20 are located in the cytosolic domain, immediately downstream of the transmembrane segment. However, the function of KFA was unknown until SIRT6 was discovered as the deacylase for TNF-α. SIRT6 deacylates TNF-α to promote its secretion (23), while fatty acylated TNF-α goes to the lysosome instead of to the plasma membrane (294). Another defatty-acylation substrate of SIRT6 is a small GTPase, R-Ras2. Ras proteins exert their effects at cellular membranes, where they interact with and activate effector proteins in various cell signaling events. Ras proteins typically have two membrane-targeting signals at the C-terminal hypervariable regions: cysteine farnesylation and cysteine palmitoylation. Additionally, they have a polybasic region containing multiple lysine residues. R-Ras2 is fatty acylated on lysine residues in the C-terminal polybasic region. KFA promotes the plasma membrane localization of R-Ras2 and its interaction with phosphatidylinositol 3-kinase, leading to activated Akt and increased cell proliferation (295). This activity may contribute to the tumor suppressor role of SIRT6.

Following the discovery of SIRT6's defatty-acylation substrates, K-Ras4a and RalB were identified as SIRT2 substrates in mammalian cells (296, 297). K-Ras4a contains KFA on three lysine residues at the C-terminal polybasic region (296). KFA also promotes K-Ras4a plasma membrane localization; however, the functional effect is different from that for R-Ras2.

SIRT2-mediated lysine defatty-acylation promotes endomembrane localization of K-Ras4a, enhances its interaction with A-Raf, and promotes cellular transformation (296). KFA is prevalent for the Ras superfamily of proteins. In a GTPase screening study, four out of nine small GTPases contained KFA. For example, KFA promotes the plasma membrane localization of RalB, which affects cancer cell migration (297). A detailed kinetic and structural study revealed that SIRT2 has a latent hydrophobic cavity that can accommodate KFA (298). This ability to accommodate KFA has enabled the development of thiomyristoyl-lysine analogs as potent inhibitors for SIRT2 (213, 299).

HDAC11 is an efficient lysine defatty-acylase with a defatty-acylase activity >10,000-fold more efficient than its deacetylase activity, which is essentially undetectable in vitro (19). This is unique among the defatty-acylases, as the sirtuin defatty-acylases also catalyze deacetylation in vivo. HDAC11 catalyzes the defatty-acylation of SHMT2. Although more commonly known as a metabolic enzyme, SHMT2 also recruits a complex to deubiquitinate and stabilize the type I interferon (IFN) receptor. Thus, HDAC11-mediated SHMT2 deacylation promotes type I IFN receptor ubiquitination and decreases its cell surface expression. Correspondingly, HDAC11 depletion increased type I IFN signaling in both cell lines and mice (19).

In contrast to the multiple defatty-acylases known in mammalian cells, so far only two mammalian writers of KFA have been reported, N-terminal glycine myristoyltransferase 1 and 2 (NMT1 and NMT2) (16). Most known writers of lysine fatty acylation are from bacteria. For example, HlyC (291) from pathogenic *Escherichia coli* myristoylates HlyA, the pore-forming RTX toxin, and this process depends on acyl carrier protein (300). After secretion, HlyA binds to mammalian cell membranes, oligomerizes to form pores, disrupts cellular activities, and causes cell lysis. HlyA is acylated heterogeneously with saturated 14- (68%), 15- (26%), and 17-carbon (6%) amide-linked side chains on K564 and K690 residues. Both sites are required for in vivo toxin hemolytic activity (301, 302). A fluorescence resonance energy transfer study suggested that KFA is important for HlyA oligomerization because it promotes protein-protein interaction (303). KFA is essential and prevalent in the pore-forming RTX toxin family. CyaC from *Bordetella pertussis*, RtxC from *Kingella kingae*, and ApxC from *Actinobacillus pleuropneumoniae* share sequence similarity with HylC and also fatty acylate and activate pore-forming toxins (304–306).

The RID domain in MARTX toxin was recently reported to be a protein lysine fatty acyl transferase. MARTX is a multifunctional autoprocessing RTX exotoxin from *Vibrio cholerae*. During pathogen-host interactions, MARTX toxins are secreted and insert themselves into the host-cell plasma membrane, where they are autocatalytically cleaved to release multiple effector domains (including the RID domain) into the cytosol of host cells (307).

IcsB is an effector from *Shigella* species that was recently reported to fatty acylate a battery of host proteins. While IcsB and RID share sequence similarities, they translocate to host cells through distinct mechanisms. IcsB is delivered directly from *Shigella* to the host-cell cytosol through the type III secretion system, a protein appendage with a needle-like structure. Chemical proteomic profiling identified approximately 60 host targets modified by IcsB during *Shigella* infection, most of which are membrane-associated proteins bearing a lysine-rich polybasic region, including members of the Ras, Rho, and Rab families of small GTPases (308). Knockout of CHMP5, one of the IcsB substrates and a component of the ESCRT-III complex, affects the ability of *Shigella flexneri* to escape from host autophagy (308).

The first mammalian fatty acyl transferases identified were the human enzymes NMT1 and NMT2 (16). NMT1 and NMT2 are well known to transfer myristoyl groups to the N-terminal glycine of proteins. Structural studies suggested that the ε-amine of lysines within the XKXXS sequence at protein N termini could mimic the α-amine of the N-terminal glycine and thus undergo lysine myristoylation (Kmyr) (16). In mammalian cells, NMT1/2 modifies ADP-ribosylation factor 6 (ARF6) on K3 to promote membrane localization. Thus, NMT1/2 together with the eraser

SIRT2 form a Kmyr-demyristoylation cycle to promote the GTPase cycle of ARF6 (16). Interestingly, inhibiting either NMT or SIRT2 decreased the activity of ARF6, highlighting the importance of the cycle. This contrasts with what we know about the role of acetylation, where acetylation and deacetylation typically have opposing effects on the substrate protein.

Crotonylation

Kcr is found in histones and other nuclear proteins (261, 309–311). The crotonyl group is a four-carbon, coplanar rigid moiety. Functionally, histone Kcr is similar to histone Kac, and both promote transcription. Histone Kcr directly stimulates transcription in a cell-free system to a greater degree than Kac (312). Chromatin immunoprecipitation sequencing (CHIP-seq) analysis suggested that histone Kcr is largely associated with active chromatin (261, 312). Kcr is associated with many biological processes, including spermatogenesis (261), metabolism (311–313), cell cycle progression (311), stem cell pluripotency (17), gut microbiota (310), and human immunodeficiency virus (HIV) latency (314).

Writers and erasers of Kcr largely overlap with those of Kac. These include p300/CBP (312), MYST (315), and GCN5 (316) as writers and class I HDACs (17) and sirtuins (317) as erasers. The crotonyltransferase activity of p300 is much weaker than its acetyltransferase activity (13, 312, 315). H3K18cr was correlated with p300 peaks in CHIP-seq, consistent with the idea that p300 is the writer of H3K18cr (312). To identify the functional contributions of Kcr, a p300/CBP mutant was generated with deficient acetyltransferase and competent crotonyltransferase activity (315). This p300/CBP mutant is a functionally active transcriptional coactivator, and its expression was correlated with enhanced promoter Kcr and recruitment of DPF2, a Kcr reader (315). Similarly, a class I HDAC mutant with competent decrotonylase but impaired deacetylase activity was generated, and its overexpression selectively impaired the recruitment of Kcr reader proteins to promoters (17). HDAC1 was observed to be crotonylated, which reduced its deacetylase activity, reminiscent of HDAC1 acetylation (311).

Unlike the writers and erasers of Kcr, which prefer Kac over Kcr, readers of Kcr are several-fold more selective for Kcr over Kac. The readers include two major classes characterized by the YEATS domain (25, 26, 318) and the DPF domain (28). Crystal and NMR structures have demonstrated that the AF9 and Taf14 YEATS domains engage Kcr via a unique π-π-π stacking mechanism between the alkene moiety of Kcr and the Trp and Phe residues in the YEATS domains (25, 26, 319, 320). Crystal structures of the DPF domain in complex with H3K14cr peptide revealed that the crotonyl moiety is anchored in a hydrophobic dead-end pocket with intimate encapsulation and crotonylamide-sensing hydrogen bonding (28). Further study of these Kcr readers will help to differentiate the functions of crotonylation from those of acetylation.

Based on the role of YEATS as a Kcr reader, a series of peptide-based inhibitors of the YEATS domain have been developed by targeting the unique π-π-π stacking interaction. One of the YEATS-selective inhibitors of the transcriptional coactivator ENL engages with endogenous ENL, perturbs the recruitment of ENL onto chromatin, and synergizes with BET and DOT1L inhibition-induced downregulation of oncogenes in mixed lineage leukemia–rearranged acute leukemia (321).

Although Kcr writers and erasers are not highly selective toward Kcr (13), it is still possible to regulate crotonylation by changing the availability of crotonyl-CoA. The cellular concentration of crotonyl-CoA can be altered by genetic and environmental perturbations (such as sodium crotonate treatment) (311–313). Chromodomain Y-like protein (CDYL) decreases crotonyl-CoA levels by converting crotonyl-CoA into β-hydroxybutyryl-CoA and thus negatively regulates histone Kcr (322). This activity is intrinsically linked to the transcriptional repression function of

CDYL and is implemented during the reactivation of sex chromosome–linked genes and histone removal during spermatogenesis (322). Addition of crotonate induces the crotonyl-CoA producing enzyme ACSS2. This is associated with increased histone crotonylation and reactivation of latent HIV (314). However, this data should be interpreted with caution as crotonate may also affect Kac by inhibiting HDACs, and it could also be metabolized into other short-chain fatty acids.

Interestingly, dynamically regulated Kcr actively participates in an adaptive response to nutrient fluctuations through epigenetic regulation (318). Histone H3K9 crotonylation level has been reported to oscillate across the yeast metabolic cycle and is sensitive to the disruption of the fatty acid oxidation pathway (318). Dynamic histone Kcr contributes to the transcriptional regulation of progrowth genes in a Taf14-dependent manner (318).

Other Lysine Short-Chain Acylations

Both histone H3 and H4 are reported to have Kpr and Kbu marks that largely overlap with lysine acetylation and also function to promote transcription (260, 323–326). Not surprisingly, the writers, erasers, and readers of these modifications also overlap with those of acetylation. Kac writers (p300/CBP and MYST family proteins) could tolerate short-chain CoA as substrates (13–15, 327–330). However, p300 and KAT2A become significantly and progressively weaker with increasing acyl-chain length (13, 15), while MYST family writers (MOF, MOZ, HBO1) have comparable Kpr and Kac activities (14, 330). Kpr and Kac are recognized by an overlapping set of bromodomain-containing proteins, including the BAF and PBAF complexes. In contrast, the same complexes did not seem to recognize Kbu (325). Interestingly, butyrylation of H4K5 inhibits the binding of BRDT, another bromodomain-containing reader, to histone H4 (323, 331). Nonetheless, a subset of human bromodomains (BRD9, CECR2, and TAF1) was found to bind Kbu and Kac with similar affinities. Structural analysis of these bromodomains revealed an aromatic gatekeeper and a flexible ligand pocket that allows high-affinity binding of butyryl-lysine (332). Sirtuins and class I HDACs were reported to work as erasers for short-chain acylation (262, 324, 329, 333–340).

Khib and Kbhb are structurally distinct from other short-chain acylations in that they are branched and have a hydroxyl group that enables the modified lysine to form hydrogen bonds with other molecules. Several HATs have been reported to perform the Khib modification. Interestingly, p300 differentially regulates Khib and Kac on distinct lysine sites. Only 6 of the 149 p300-targeted Khib sites overlap with the 693 identified p300-targeted Kac sites (327, 328). Moreover, ENO1, along with other glycolytic enzymes, is modified with Khib, which upregulates ENO1 activity to a greater extent than Kac (327). These observations suggest that the roles of Khib are distinct from those of Kac. Not much is known about the erasers and readers of these modifications. Histone Khib and Kbhb are found on many proteins and are adjusted according to metabolic cues (262, 263, 339). For example, H4K8hib levels fluctuate in response to the availability of carbon, and low-glucose conditions lead to fewer modifications in budding yeast (339). During starvation, ketone bodies that contain β-hydroxybutyrate are released by the liver and serve as an energy source for other tissues. In cases of untreated diabetes, serum concentrations of β-hydroxybutyrate can be elevated up to 20-fold. This hints at a relevant and important metabolic regulatory role for Kbhb (263, 328). Indeed, histone Kbhb is significantly induced under starvation and diabetic ketosis conditions (263). This increase in H3K9Kbhb is associated with the upregulation of genes involved in starvation-responsive pathways (263). p53 also contains Kbhb at multiple sites, leading to lower levels of p53 acetylation and reduced transcriptional activity in cells (328).

Lysine lactylation (Kla) was recently reported as a transcription-activating epigenetic marker on core histones (H3, H4, H2A, and H2B) (341). Kla is regulated by endogenous production of

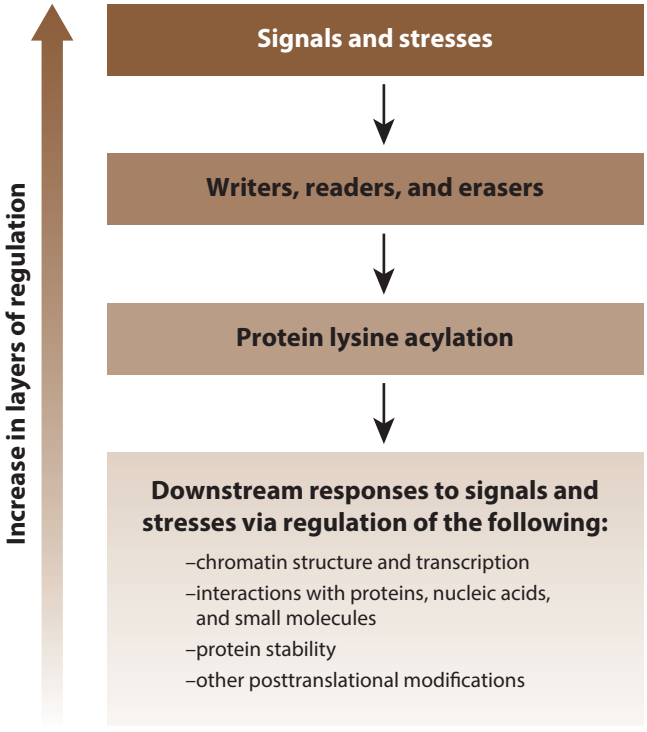

Figure 4

Regulation of the regulators of lysine acylation allows lysine acylation to mediate various cell signaling events. By changing the activities of the writers, readers, and erasers, signals and stresses could lead to changes in the lysine acylation of various proteins, allowing cells to respond to signals and stresses.

lactate, a key metabolite in many biological processes associated with the Warburg effect (341). Interestingly, in M1 macrophages where aerobic glycolysis is elevated, histone Kla activation shows a different temporal dynamic pattern from acetylation (341). The regulation and function of Kla await future investigation.

CONCLUDING COMMENTS AND FUTURE PERSPECTIVES

Learning from Acetylation to Investigate Other Acylations

It is clear that we know much more about the function of Kac than any other lysine acylation. This knowledge has now allowed us to understand the logic of using acetylation to achieve specific physiological responses to different signals or stresses (**Figure 4**). To a large extent, this knowledge is based on our understanding of how the deacylases, especially sirtuins, are regulated. It is through such regulatory mechanisms that cells are able to dynamically control Kac and mediate various signaling events, just as the regulation of protein kinases allows phosphorylation to mediate cell signaling. Our understanding of Kac is an important foundation for understanding the function of other lysine acylations. Therefore, we believe future studies on other lysine acylations should focus on the regulation of their writers, readers, and erasers, in addition to identifying more proteins that they modify and studying how the modifications affect protein function. These tasks may be difficult for the lysine acylations that share writers, readers, and erasers, but they should be feasible

for those that have distinct writers, readers, or erasers. For example, understanding the regulation of SIRT5 will likely provide important insights into Kmal, Ksuc, and Kglu. Obviously, for lysine acylations for which writers, readers, and erasers are not known, identifying the enzymes is an important first step. For example, identification of the writers of KFA and Kla is clearly needed.

Learning from Other Acylations to Understand Acetylation

While much can be learned from Kac that helps us to understand other lysine acylations, knowledge gained from other lysine acylations may also help us to understand the function of Kac. One example is the discovery of a Kmyr-demyristoylation cycle for the small GTPase ARF6. In the Kac field, researchers are used to the idea that if Kac promotes a certain function, then deacetylation should inhibit it, and vice versa. However, for ARF6, the myristoylation-demyristoylation cycle is coupled to its activity cycle, and as a consequence, both myristoylation and demyristoylation promote ARF6 activity. This is likely also to be true for acetylation-deacylation in certain cases, such as DNA damage and repair, where both HATs and HDACs have been reported to be important. Thus, learning from other acylations, and even other types of PTMs, could potentially lead to a greater understanding of Kac.

Unaddressed Questions for the Relatively New Acyl-Lysine Modifications

Many questions remain to be addressed for the relatively new acyl-lysine modifications. Some of them are very basic, such as how hydroxyisobutyryl-CoA is generated in mammalian cells. While β-hydroxybutyryl-CoA is generated through ketogenesis or fatty acid oxidation, no pathways are known in humans to produce hydroxyisobutyryl-CoA. A possible source of hydroxyisobutyrate is the microbiome, similar to propionate and butyrate, which are common by-products of bacterial fermentation and are produced in large quantities (50–100 mM) in the gastrointestinal tract of mammals (342). Therefore, our understanding of lysine acylation could potentially benefit from investigation into microbiomes and may provide a mechanism for microbiome-host interaction.

Unlike other lysine acylations, which were initially discovered on histones, no histone lysine KFA marks have been identified so far. It is plausible that histones are not fatty acylated due to the limited amount of fatty acyl-CoA present in the nucleus. However, it is possible that certain acyl-CoA synthetases could be present in the nucleus to increase the local concentration of free fatty acyl-CoAs. Therefore, it is worth exploring whether histones are also modified with long-chain fatty acyl–lysine.

Cross Talk Among Different Acylations

Several histone lysine residues are known to be modified by different acyl modifications. Kac and Ksuc sites also overlap on many mitochondrial proteins. Thus, one lysine site could be modified by multiple acyl groups, and the different acylation events may affect each other. An interesting possibility is that different acyl-lysine modifications could cross talk by affecting the sirtuins. This may depend on the nature of the acyl-lysine modifications. For example, acetyl-lysine is not likely to inhibit SIRT5 because SIRT5 prefers to bind succinyl-lysine. Similarly, succinyl-lysine is not likely to inhibit SIRT3. However, long-chain fatty acyl–lysine may affect the deacetylation activity of sirtuins (SIRT1–3, SIRT6, and SIRT7), because the binding affinity of these sirtuins for long-chain fatty acyl–lysine is much higher than that for acetyl-lysine. When there are high levels of long-chain fatty acylation, deacetylation of substrates by sirtuins might be inhibited. A high-fat diet is known to increase protein acetylation levels. One possible explanation is that a high-fat

diet could increase fatty acylation levels, thus inhibiting sirtuin-catalyzed deacetylation. It would be interesting to examine the possible interactions among different lysine acylation events.

Important Questions Regarding Acetylation

Even for the most well-studied modification, Kac, many questions remain. Part of the reason is that many biological processes regulated by Kac, such as transcription and DNA damage repair, are very complex. Our understanding of these complex processes is still preliminary and evolving. The phase separation or phase condensation model for transcription is a fairly recent development in the field and is certain to stimulate new research on Kac, as well as other lysine acylations. Many mitochondrial proteins are acetylated and regulated by SIRT3, but whether the mitochondrial acetylation events are enzymatic or nonenzymatic still needs to be investigated. Furthermore, although we touched on many regulatory mechanisms of sirtuin deacetylases, much remains to be understood about these regulatory mechanisms and how they are connected to known biological functions. One obvious example is regulation by miRNA. While many miRNAs have been reported to regulate the sirtuins, in most cases, we do not understand the logic for using that regulatory mechanism. Thus, to the curious mind, much remains to be explored regarding Kac.

While we did not touch on it in this review, Kac has long been used as a therapeutic target for several human diseases, including cancer and neurological diseases. Many labs are also interested in exploring its potential for regulating health span and longevity. We believe that through continued research and improved understanding, we can develop better therapeutics targeting Kac and other lysine acylations.

DISCLOSURE STATEMENT

H.L. is a founder and consultant for Sedec Therapeutics. M.W. is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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