Review

MOLECULAR METABOLISM

Metabolic intermediates — Cellular messengers talking to chromatin modifiers

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ABSTRACT

Background: To maintain homeostasis, cells need to coordinate the expression of their genes. Epigenetic mechanisms controlling transcription activation and repression include DNA methylation and post-translational modifications of histones, which can affect the architecture of chromatin and/or create 'docking platforms' for multiple binding proteins. These modifications can be dynamically set and removed by various enzymes that depend on the availability of key metabolites derived from different intracellular pathways. Therefore, small metabolites generated in anabolic and catabolic processes can integrate multiple external and internal stimuli and transfer information on the energetic state of a cell to the transcriptional machinery by regulating the activity of chromatin-modifying enzymes.
Scope of review: This review provides an overview of the current literature and concepts on the connections and crosstalk between key cellular metabolites, enzymes responsible for their synthesis, recycling, and conversion and chromatin marks controlling gene expression.
Major conclusions: Whereas current evidence indicates that many chromatin-modifying enzymes respond to alterations in the levels of their cofactors, cosubstrates, and inhibitors, the detailed molecular mechanisms and functional consequences of such processes are largely unresolved. A deeper investigation of mechanisms responsible for altering the total cellular concentration of particular metabolites, as well as their nuclear abundance and accessibility for chromatin-modifying enzymes, will be necessary to better understand the crosstalk between metabolism, chromatin marks, and gene expression.

Keywords Metabolism; Histone modifications; Gene expression; Metabolic enzymes

1. INTRODUCTION

The term 'epigenetics' was originally coined by Waddington in 1942 [1]; however, the usage and definition of 'epigenetics' has changed throughout the years. The Greek prefix 'epi-' (meaning 'over' or 'above') emphasizes phenomena that reach beyond well established genetic (DNA sequence based) mechanisms. In the same notion, in 2008, an 'epigenetic trait' was defined as a 'stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence' [2]. Therefore, one of the key implications of 'epigenetics' is that a cell can preserve a memory of past states and signals, triggering its future behavior, in the absence of both the initial signal and alterations in the DNA sequence.
In multicellular organisms epigenetic factors can enable cells to activate or repress particular sets of genes. There are several mechanisms that are implicated in mediating inheritance and maintenance of such gene expression states. The most common mechanisms, and the only ones discussed in this review, are covalent modifications of chromatin. This review focuses on a new aspect of 'epigenetics', the link between the metabolic state of a cell and chromatin architecture. In particular it discusses the metabolites, generated and converted in various physiological pathways, which are of great importance to many epigenetic 'writers' and 'erasers', namely: S-adenosylmethionine (SAM), α-ketoglutarate (α-KG), succinate, fumarate, acetyl-CoA, short chain acyl-CoAs and NAD^+. We summarize the existing body of knowledge on metabolism of the aforementioned compounds, their subcellular localization and impact on chromatin modification and, thus, gene expression. Although many aspects of the links between metabolism and epigenetics have been excellently reviewed elsewhere (see for example: [3—11]), here we touch on frequently neglected aspects of subcellular distribution of metabolites and enzymes as well as on kinetic parameters of chromatin-modifying enzymes and on physiological concentrations of key cofactors. Finally, we highlight the existing gaps in the field and suggest potential future directions.

2. EPIGENETIC MECHANISMS REGULATING CHROMATIN FUNCTIONS

Early studies in mouse revealed that a modification of DNA itself, DNA methylation, could act as an 'epigenetic' mark, for example in X chromosome inactivation [12]. The discovery of enzymatic semi-conservative propagation of such DNA methylation patterns at CpG sites after replication and mitotic division gave the first insights into how such 'epigenetic' information could be maintained through cell divisions. Attention is now being focused on the possible transgenerational transmission of these patterns, the contribution of DNA methylation to

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gene silencing, mechanisms initiating or preventing methylation on fully unmethylated sites, and the enzymes catalyzing this modification [13]. DNA methylation patterns are not static and can be highly dynamic; for example, during preimplantation development, major changes in DNA methylation patterns occur in the mammalian genome. In the preimplantation state, methylation marks on both the maternal and paternal DNA are largely erased and subsequently reestablished *de novo*. This requires another set of enzymes, different from the ones responsible for maintenance of CpG methylation in somatic cells.

Eukaryotic DNA exists as a complex of DNA and proteins, which are collectively the chromatin. The most abundant proteins in chromatin are histones, discovered by Kossel in the nineteenth century [14]. Histones, the main structural components of eukaryotic chromatin, are highly basic proteins that, together with the DNA wrapped around them, form nucleosomes. Initial suggestions that histones are merely general repressors of gene expression were refined after a breakthrough discovery by Allfrey and Mirsky in 1964; they showed that histones can be modified by lysine acetylation and that this acetylation is positively correlated with gene activation [15]. Since that time, interest in histone modifications and their role in gene expression have been growing. The functional significance of various chemical groups attached to histones became clearer when the structure of the nucleosomal core particle was determined [16]. The nucleosomal core particle is composed of 147 bp of DNA wrapped around a histone octamer consisting of 2 copies of each of the core histones: H2A, H2B, H3, and H4, with their globular domains forming a 'spool' for the DNA and the terminal tails protruding from the core of nucleosome. Histone H1, also known as the linker histone, binds to the linker DNA region between nucleosomal core particles.

Chromatin architecture and dynamics contribute significantly to gene expression. A wide range of histone post-translational modifications (PTMs) plays an important role in establishing various chromatin states. This may be achieved by both changing the physical properties of nucleosomes and by recruiting different binding proteins that then exert effects on downstream events such as transcription. Some of the most studied histone PTMs are phosphorylation, methylation, acetylation, and ubiquitylation, thoroughly reviewed in [17—20]. Additionally, novel histone modifications are continuously being identified. It is now known that histone PTMs contribute to the mechanisms by which DNA-sequence specific transcription factors and additional transcriptional regulators modulate expression and silencing of genes. One of the first examples supporting this notion came from the work on a yeast protein Gcn5, a component of the SAGA complex, previously associated with active transcription. Gcn5 was shown to possess histone acetyltransferase activity and hence to act as a 'writer' of histone modifications [21]. Acetylation of lysines within N-terminal histone tails is highly enriched at the promoter regions of genes and is generally associated with chromatin decompaction, promoting high levels of gene expression. The function of histone methylation on lysines and arginines and its contribution to gene expression depends on the modified residue. For example, mono- and tri-methylations are found to be enriched on active promoters (e.g. H3K4me3), active enhancers (H3K4me1), inactive promoters (e.g. H3K27me3) and coding regions of active genes (e.g. H3K36me3). Most histone modifications are reversible and can be removed by so-called 'erasers'. The combined range of histone PTMs and DNA modifications can constitute particular epigenetic patterns that can be specifically recognized and bound by so-called 'reader' proteins. Some of these readers have their own histone-modifying activity, where their role can be to recognize and bind a particular PTM, introduce the same mark onto the adjacent nucleosome and thus propagate a particular modification in a defined region of the genome [22]. Other 'readers' can be e.g. subunits of ATP-dependent chromatin-remodeling complexes that modulate gene expression by ejecting or 'sliding' nucleosomes. Combinatorial patterns of histone PTMs are crucial determinants of the state and architecture of chromatin — that can be accessible to transcription, DNA repair and replication or compacted and usually devoid of transcribed genes.

Apart from recruiting 'reader' proteins, some histone PTMs have been shown to affect the structure or dynamics of chromatin on their own. These effects are mainly observed for modifications of residues within globular domains of histones. Acetylation of H3K64 by p300 destabilizes nucleosomes and can promote histone eviction and transcription [23]. Similarly, acetylation of H3K122, a residue that physically interacts with DNA and thus has been anticipated to affect nucleosome stability, stimulates transcription of *in vitro* assembled chromatin [24,25]. Acetylation of H4K16 is so far the only tail modification that directly affects chromatin structure [26] — when incorporated into *in vitro* reconstituted nucleosomal arrays, this modification was shown to prevent chromatin from forming higher order structures.

Since covalent modifications of chromatin components underlie gene expression programs, the activity of the enzymes responsible for placing these modifications and their removal can be a crucial factor controlling transcription. In the last few years it has become evident that small metabolic intermediates can be common denominators between two elementary biological processes: energy metabolism and gene regulation. Cells in a particular metabolic condition established by available nutrients, external stimulation and their intrinsic metabolic status, produce specific sets of metabolites, many of which can serve as cofactors or inhibitors of chromatin-modifying enzymes and hence regulate their activity or specificity (Figure 1).

3. SAM AS A COMMON COFACTOR FOR METHYLATION OF CHROMATIN

3.1. DNA and RNA methylation as epigenetic marks

Methylation of DNA was first reported in 1963 [27]. Since the discovery that hypo-methylation of particular genes is associated with some human cancers, its role in normal human development, aging and tumorigenesis has been thoroughly explored [28,29]. Enzymatic transfer of a methyl group to the C-5 position of the cytosine is catalysed by DNA methyltransferases (DNMTs), of which are three main ones in mammals: DNMT3A, DNMT3B and DNMT1. While DNMT3A/B are mainly responsible for setting *de novo* methylation patterns (e.g. in early embryo development) and are able to methylate non-methylated cytosines, DNMT1 — the most abundant methyltransferase — acts predominantly on hemi-methylated CpG dinucleotides and thus provides stability of methylation patterns after DNA replication through cell division, making DNA methylation a truly 'epigenetic' mark [30,31].

From the more than 100 various covalent modifications of RNA that have been identified, methylation of adenine at the N-6 position (m^6A) is the most prevalent in eukaryotic mRNA [32]. Although studies have reported different effects of m^6A on RNA functions and correlations between alterations in m^6A patterns and various human diseases, including many cancers, its functional role is just emerging and needs further investigation [33].

3.2. Histone methylation can repress or activate transcription

The catalogue of histone methylation states is ample as methylation can occur on lysine (Lys), arginine (Arg) and histidine (His) residues. Moreover each Lys residue can carry one, two or three methyl groups (mono-, di- and tri-methylation, respectively) and each arginine can be mono- and symmetrically or asymmetrically di-methylated. The most

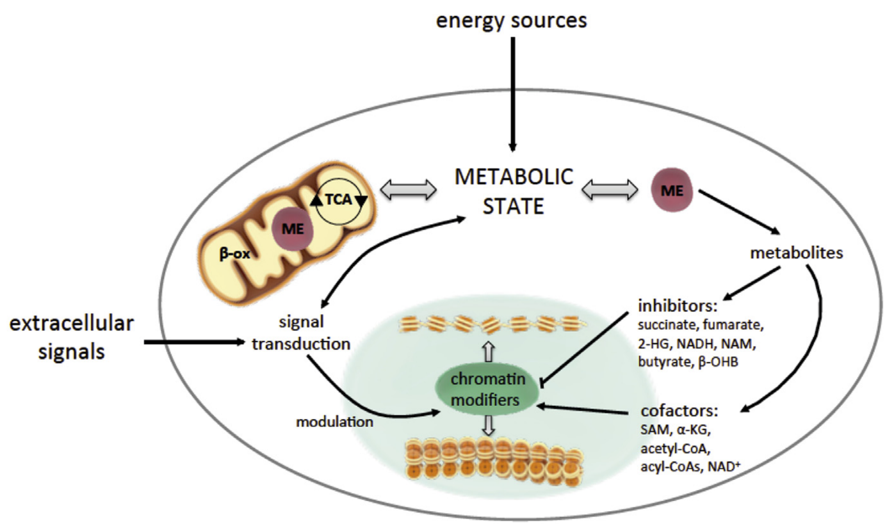

Figure 1: Crosstalk between metabolism and chromatin modifications. Cells supplied with external energy sources are in a metabolic state with distinct intermediary metabolites generated by metabolic enzymes (ME). Many of these metabolic intermediates can serve as cofactors or inhibitors of chromatin modifiers — such as 'hubs' collecting intra- and extracellular signals and transferring them to the chromatin level and thus affecting transcriptional events; 2-HG — 2-hydroxyglutarate, NADH — reduced form of nicotinamide adenine dinucleotide; NAD^+ - oxidized form of nicotinamide adenine dinucleotide, NAM — nicotinamide, TCA — tricarboxylic acid cycle, α-KG — α-ketoglutarate, β-OHB — β-hydroxybutyrate, β-ox — β-oxidation.

studied histone methylation sites include: H3K4, H3K9, H3K27, H3K36, H3K79, H4K20, H3R2, H3R8, H3R17, H3R26 and H4R3 [18].
There are two major families of histone methyltransferases that set these modifications: Lys- and Arg-specific methyltransferases. Although describing the domain structure, specificity and preference of all the members of each family is beyond the scope of this article, the common feature of all aforementioned histone methyltransferases, as well as DNMTs, is necessity of the cofactor SAM to perform their enzymatic function (Figure 2A). SAM is synthesised directly from methionine (Met) by S-adenosylmethionine synthetases (see below). For humans Met is an essential amino acid, however it can be recycled in the methionine cycle from S-adenosylhomocysteine (SAH) via homocysteine (homoCys) with a supply of methyl-tetrahydrofolate (methyl-THF) derived from other amino acids (threonine (Thr), glycine (Gly), serine (Ser)) and folic acid. In the liver and kidney Met can also be synthesised from homo-cysteine with betaine as a methyl donor [34].

3.3. Metabolic impact on DNA methylation

One interesting example of a connection between diet and DNA methylation observed in mice is the effect of folate in the mothers' diet on the phenotype of their offspring. Insertion of a repetitive element, an intra-cisternal A particle (IAP) retrotransposon, into the 5′ end of the agouti gene results in the agouti viable yellow phenotype (A^{vy}), affecting the colour of the mice. The penetrance of the A^{vy} phenotype depends on the CpG methylation level of the IAP retrotransposon; high methylation correlates with low expression of A^{vy} (agouti-coloured mice) while low methylation correlates with high expression of A^{vy} (yellow-coloured mice). Females whose diet was supplemented with methyl donors gave birth to offspring with increased eumelanic mottling (agouti/black areas on yellow background) compared to the control group showing that dietary intake of methyl donors can regulate the state of chromatin marks [35].
Studies in mouse embryonic stem cells (mESCs) provide another example linking the cellular metabolic state, via concentrations of compounds involved in the methionine cycle, to chromatin modifications. Mitochondrial threonine dehydrogenase (*Tdh*) generates Gly and thus supplies the one-carbon metabolism with methyl groups. Depletion of Thr from the culture medium or knockdown of *Tdh* decreases SAM concentrations as well as the levels of di- and tri-methylation of H3K4. Moreover, mESCs show higher expression levels of *Tdh* in comparison to differentiated cells [36], and Thr restriction leads to slowed growth and increased differentiation. Remarkably, other modifications like H3K4me1, H3K9me3, H3K27me3, H3K36me3 and H3K79me3, were not affected by the knockdown, which could imply distinct sensitivity of different histone Lys methyltransferases (KMTs) for SAM levels [37]. It is not known if Thr metabolism has the same impact on histone Lys methylation levels in human ESC where *TDH* is a non-functional transcribed pseudogene [38] and Thr conversion is catalysed by L-Ser/Thr dehydratase, whose role in one-carbon metabolism has not been fully investigated. Nevertheless, Met restriction leads to a reduction in H3K4me3 levels across a panel of human cancerous cell lines, clearly demonstrating the importance of metabolism of particular amino acids in regulatory histone methylation [39].

3.4. Methionine adenosyltransferases are required for SAM biosynthesis

Synthesis of SAM, a cofactor for all methylation reactions, requires Met and ATP and is catalysed by methionine adenosyltransferases (MATs). There are 3 MAT isozymes in mammals (Figure 2E). Two genes encode homologous, but different, MAT catalytic subunits. *MAT1A*, mostly expressed in liver, encodes the α1 subunit found in two native MAT isozymes, which are either a tetramer (MAT I) or dimer (MAT III) of this subunit. *MAT2A* is expressed in many tissues including brain, kidney, testis, lymphocytes, foetal liver and, to a lesser extent, adult liver. The catalytic subunit it encodes (α2) is found in a native MAT isozyme (MAT II) associated with the catalytically inactive, regulatory subunit β encoded by *MAT2B*.
Impaired enzymatic activity of MATs has a profound effect on histone methylation in many organisms. Depletion of MAT2A in immortalised mouse embryonic fibroblasts (iMEF) significantly reduces tri-methylation levels of H3K4 and H3K9 on a genome-wide scale without affecting the levels of mono- and di-methylation of these sites

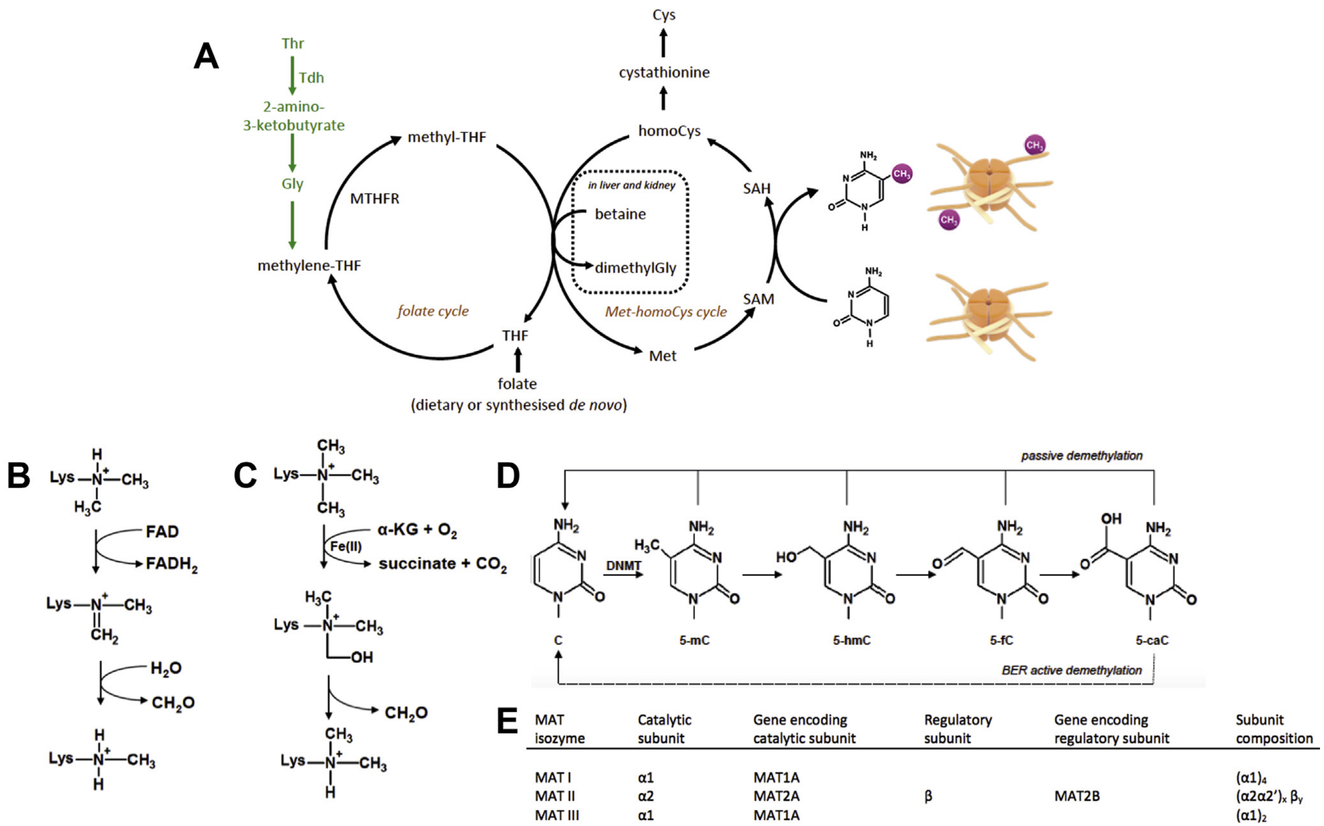

Figure 2: Metabolites are involved in methylation and demethylation of chromatin; a. The interplay between folate and Met-homoCys cycles. Dietary or *de novo* synthesized folate and (in organisms expressing Tdh — in green) Thr supply the folate cycle with THF and methylene-THF, respectively. This cycle generates methyl-THF subsequently used as a donor of the methyl group in the Met-homoCys cycle. In liver and kidney betaine, instead of methyl-THF, serves as a methyl donor; Cys — cysteine, homoCys — homocysteine, Gly — glycine, Met — methionine, MTHFR — methylene-THF reductase, SAH — S-adenosylhomocysteine, SAM — S-adenosylmethionine, Tdh — threonine dehydrogenase, THF — tetrahydrofolate, Thr — threonine; b. The FAD dependent demethylation of Lys occurs through the two electron oxidation of an amine by flavin followed by the hydrolysis of an iminium ion; c. The Fe(II) dependent demethylation of trimethyl-Lys occurs through an Fe(II), α-KG, and O_2 derived radical oxidation of the methyl C—H bond; d. 5-mC conversion by TET enzymes. All intermediary products could be passively removed e.g. by DNA replication; 5-mC — 5-methylcytosine, 5-hmC — 5-hydroxymethylcytosine, 5-fC — 5-formylcytosine, 5-caC — 5-carboxycytosine, BER — base excision repair; e. Summary of human methionine adenosyltransferase genes and gene products.

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[40]. In *Caenorhabditis elegans*, knockdown of *sam1* (homologue of mammalian *MAT2A*) reduces H3K4me3 levels [41]. Simultaneous knockdown of *sam3* and *sam4* leads to a reduction in H3K9me3, H3K27me3 and H3K36me3 but does not affect H3K4me3 [42]. Similarly, H3K4me3 but not H3K4me1, H3K4me2, H3K36me3 nor H3K79me3 was reduced upon knockout of the yeast homologues of *MAT*s – *sam1* and *sam2* [43]. Taken together, these findings raise the possibility that (in particular tissues) the activity of specific subsets of KMTs could depend on MAT activity. Interestingly it was shown that in mammals the recruitment of MATII to the cyclooxygenase 2 (*COX-2*) locus provides a local supply of SAM for the H3K9 methyltransferase SETDB1 and represses the expression of *COX-2* [40]. In addition to these functional links between KMTs and MATs, studies in yeast identified a protein complex SESAME consisting of multiple metabolic enzymes including SAM synthetases and the Set1 KMT catalysing H3K4 methylation [43]. Thus, recruitment of the SESAME complex containing both KMT and SAM synthetases to target genes brings both chromatin modifiers and the enzymes responsible for generating the required cofactor in close proximity, creating a local 'chromatin niche' (or 'chromatin microdomain') with increased local concentration of SAM used for histone methylation [6]. Whether the same mechanism also exists in higher eukaryotes is still not known.

4. METABOLITES CRUCIAL FOR CHROMATIN DEMETHYLATION

4.1. Lysine-specific histone demethylase 1 and 2 (LSD)

Methylation of Lys residues was long believed to be irreversible, but finally in 2004, the first Lys demethylase (KDM) was identified and named LSD1 (Lys-specific demethylase 1) [44]. It is a flavin-dependent amino oxidase specifically demethylating mono- and di-methylated H3K4 (Figure 2B). Recently, LSD2, another flavin-dependent KDM acting on the same residue, has been identified in mammals [45]. The catalytic mechanism of LSD1/2 involves forming an imine intermediate and thus does not allow them to remove methyl groups from tri-methylated Lys.

4.2. Fe^{2+} and α-ketoglutarate dependent dioxygenases can also demethylate histones

The largest class of histone demethylases are Jumonji C (JmjC) domain-containing demethylases, which belong to the bigger family of Fe^{2+} and α-KG dependent dioxygenases (2-OGDO) and are grouped into several subfamilies. The catalytic mechanism is different from the previously mentioned amino oxidases. JmjC demethylases can remove methyl groups from mono-, di- and tri-methylated Lys, forming intermediary hydroxymethyl-Lys (Figure 2C).

4.3. Ten-eleven translocation enzymes modify DNA

For many years there was a notion that the loss of methyl marks on C-5 cytosine occurred through 'passive dilution' during DNA replication and DNA repair processes. The discovery that Ten-eleven translocation (TET) enzymes, previously associated with translocations in some types of cancer, catalyse iterative conversion of 5-methylcytosine (5-mC) shed new light on DNA demethylation mechanisms (Figure 2D) [46]. The catalytic mechanism of TET enzymes is similar to JmjC demethylases – they use oxygen to decarboxylate α-KG, generating a high-valent iron oxide converting 5-methyl- (5-m-) to 5-hydroxymethyl- (5-hm-) and further to 5-formyl- (5-f-) and 5-carboxycytosine (5-caC), which can subsequently be removed through base excision repair (BER) [47]. Apart from serving as an intermediary product in the demethylation process, 5-hmC itself has been suggested to be a functional DNA modification and can be highly abundant in certain tissues such as mouse brain [48].

4.4. Role of TCA cycle metabolites in chromatin demethylation

The involvement of α-KG in many cellular processes such as amino acid and protein synthesis, tricarboxylic (TCA) cycle and nitrogen transport, as well as its role in stabilizing immune system homeostasis and modulating senescence, makes this compound a promising candidate as a key sensor of the metabolic state of a cell and of the whole organism [49]. Availability of α-KG for 2-OGDO enzymes could regulate their activity on methylated chromatin. One observation supporting this notion comes from a study in mESCs where decreasing the α-KG/succinate ratio by manipulating medium composition led to a global increase in H3K27 and DNA methylation levels [50]. In this cellular model, maintaining the α-KG pool favours active demethylation of repressive marks, contributing to a suppression of cellular differentiation.

Adding to the complexity is the fact that succinate and fumarate, other metabolites of the TCA cycle, can act as competitors of α-KG and thus inhibit the activity of multiple 2-OGDO including TET1/2 and the JmjC domain-containing histone demethylases KDM4A, KDM4D and KDM4DL [51]. Tumors with impaired activity of succinate dehydrogenase (*SDH*) or fumarate hydratase (*FH*) (converting succinate to fumarate and fumarate to malate, respectively) accumulate up to millimolar concentrations of succinate and fumarate [52,53]. HEK293T cells ectopically expressing tumor-derived mutants of *SDH* and *FH* showed decreased activity of KDMs manifested by increased H3K4me1, H3K4me3, and H3K9me2 levels (in comparison to cells expressing wild type *SDH* and *FH*), which was accompanied by up-regulation of several *HOXA* genes. Isocitrate dehydrogenase (IDH) is the enzyme catalysing reversible conversion of isocitrate to α-KG. In humans, there are 3 isoforms of IDH. IDH1 is localized in the cytosol and in peroxisomes while IDH2 and IDH3 are found mainly in mitochondria. IDH1 and IDH2 are $NADP^+$-dependent enzymes that function as homodimers. They show high structural similarity and perform analogous functions in different subcellular compartments. IDH3 differs structurally from the two other isoforms, working as heterotetramer formed by the 2 α and 2 β subunits. IDH3 requires NAD^+ as a cofactor and has a well established role in the TCA cycle [54]. Interestingly, gain-of-function mutations in the catalytic site of *IDH1/2* were observed in some gliomas and acute myeloid leukaemia (AML) [55]. These mutations resulted in the ability of the enzymes to reduce α-KG to the R enantiomer of 2-hydroxyglutarate (R-2HG), which is a competitive inhibitor of JmjC KDMs and TET demethylases. These neomorphic mutations correlate with an increase in global 5-mC levels [56].

Taken together, impaired chromatin marks, and subsequently gene expression, in some types of cancers can be caused by dysregulation of TCA cycle enzymes affecting the physiological ratio of α-KG to 2-OGDO inhibitors.

5. MECHANISMS OF HISTONE ACETYLATION

Protein acetylation normally occurs in two distinct forms. In humans, more than 80% of proteins become co-translationally (and, to a lesser extent, post-translationally) acetylated at their α-amino group by N-terminal acetyltransferases. The second major type of acetylation occurs on the ε-amino group of Lys residues. The first-described evidence of Lys acetylation as a post-translational modification (PTM) comes from studies carried out in 1964, when this mark was found on histone tails isolated from calf thymus nuclei [15]. Since then, many proteins carrying acetyl marks on Lys residues have been identified. While N-terminal acetylation is considered to be largely irreversible, Lys acetylation is highly and dynamically regulated by the competing activity of Lys acetyltransferases (KATs) and Lys deacetylases (KDACs). Up to now, acetyl groups have been found on more than 60 histone Lys residues. In the epigenetics field, therefore, KATs and KDACs are often called HATs and HDACs (for 'histone acetyltransferases' and 'histone deacetylases' respectively) [57]. The majority of acetylated Lys within core histones is located in their N-terminal tails and is usually correlated with active transcription. These modifications include: K5 in H2A; K5, K12, K15, and K20 in H2B; K4, K9, K14, K18, K23, and K27 in H3; K2, K8, K12, and K16 in H4 [58]. In recent years, the role of acetylation within histone globular domain in regulation of transcription has also been reported [23,25,59]. At physiological pH, which is lower than the pI of Lys ($pI_{Lys} = 9.74$), ε-amino groups are positively charged. Addition of an acetyl group directly neutralizes the charge, potentially disrupting electrostatic interactions with the negatively charged DNA and between adjacent nucleosomes [26]. This phenomenon can result in 'opening' of the chromatin making it more permissive to transcription. Indirect effects of acetylation rely on the proteins or protein complexes interacting with single modifications or combinations of modifications. Acetyl groups are recognized and bound mainly by bromodomain-containing proteins and protein complexes commonly associated with promoting gene activation [60]. In humans there are 46 cytoplasmic and nuclear proteins containing a total of 61 bromodomains (BRDs) [61]. Among chromatin-related BRD-containing proteins there are HATs (KAT2A, KAT2B, BRD9), histone methyltransferases (ASH1L, MLL), helicases (SMARCA), subunits of ATP-dependent chromatin remodeling complexes (BAZ1B), transcriptional coactivators (TRIMs, TAFs), transcriptional mediators (TAF1) and nuclear scaffolding proteins (PBRM1) [62]. Therefore, the acetylation pattern of histones can drive various downstream chromatin-related events, contributing to changes in the transcription of particular genes.

5.1. Acetyl-CoA levels regulate histone acetylation state

All KATs utilize acetyl-CoA as a donor of acetyl groups for protein acetylation. Based on kinetic and binding parameters of mammalian KATs, many of their activities should be sensitive to physiological fluctuations of acetyl-CoA [63]. Of note, many KATs are inhibited by the product of the reaction, coenzyme A (CoA), and bind both acetyl-CoA and CoA with similar affinities [63]. Consequently, the ratio of these metabolites and not solely the concentration of acetyl-CoA might be the critical determinant for overall histone acetylation levels. Acetyl-CoA is a key intermediate involved in many metabolic pathways including cellular respiration, fatty acid, steroid and amino acid metabolism, synthesis of ketone bodies and neurotransmitters and has also been implicated in affecting the AMPK signaling pathway. Acetyl-CoA can be synthesized in mitochondria and the cytosol, and, more recently, synthesis in the nucleus has also been reported (see below) (Figure 3A). In mitochondria, acetyl-CoA is a product of oxidative decarboxylation of pyruvate by pyruvate dehydrogenase (PDH) and can be used to fuel the TCA. Since there is no transporter of acetyl-CoA across mitochondrial membranes, it is first converted to citrate and subsequently shuttled to the cytosol, where the reverse reaction to generate acetyl-CoA again is catalyzed by ATP-citrate lyase (ACL). An alternative source of cytosolic acetyl-CoA is the conversion of acetate by acetyl-CoA synthetase (ACSS2). In line with this, an impact of depletion of PDH, ACL, and ACSS2 on histone acetylation has been observed in mammalian cells. Knockdown of ACSS2 in a murine neuronal cell culture model leads to decreased level of H3K9ac and H3K27ac [64]. Similarly, knockdowns of ACL in the human line HCT116 and of the E1α1 subunit of PDH in HeLa S3 cells have a profound effect on global acetylation of H2B, H3, and H4 and H3K18ac, respectively [65,66]. The fact that knockdowns of various enzymes affect histone modifications in different ways implies that depending on the cell line, tissue, or metabolic state of a cell, the major source of acetyl-CoA used for histone acetylation can be different.

Although acetyl-CoA can diffuse through nuclear pores, under specific cellular conditions PDH, ACL and ACSS2 can transiently localize to the nucleus and provide a local supply of acetyl-CoA for histone acetylation

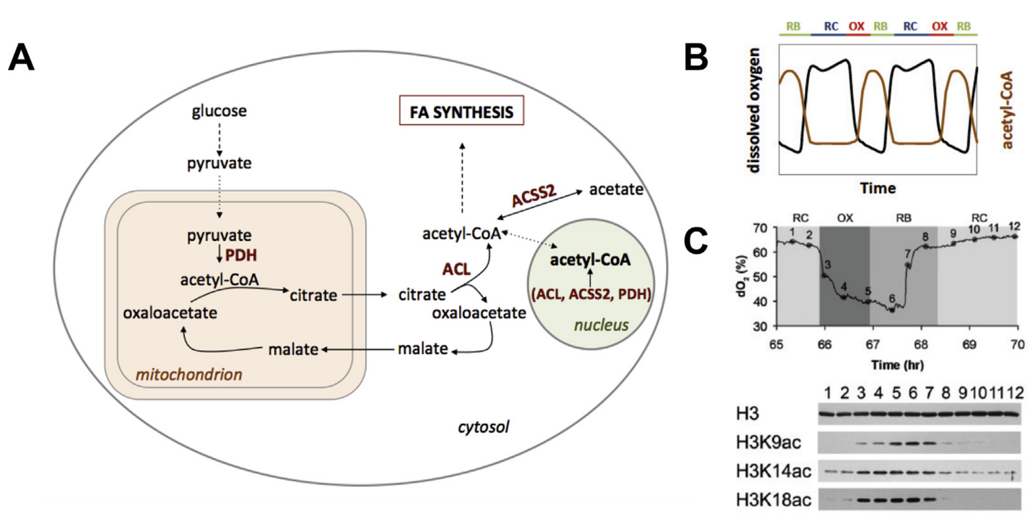

Figure 3: Acetyl-CoA is a key metabolite linking metabolism and chromatin state. a. Subcellular pools of acetyl-CoA generated by different enzymes; ACL — ATP-citrate lyase, ACSS2 — acyl-coenzyme A synthetase short-chain family member 2, PDH — pyruvate dehydrogenase; dotted lines — passive diffusion, dashed line — multi-step process; b. Fluctuations of oxygen consumption and intracellular acetyl-CoA concentration during yeast metabolic cycle (YMC); c. Western blot analysis of cell extracts prepared from samples collected at indicated time points of YMC shows the induction of acetylation of selected H3 Lys residues upon entry into RB phase. Histone acetylation is tightly correlated with the peak of intracellular acetyl-CoA concentration; OX — oxidative phase, RB — reductive binding phase, RC — reductive charging phase; c. reprinted from [68] with permission from the authors and ELSEVIER.

[64,65,67]. Such a mechanism increases the local concentration of acetyl-CoA and could mediate more efficient histone acetylation even if the total cellular concentration of acetyl-CoA does not change significantly.

5.2. Physiological fluctuations of acetyl-CoA levels

The first clear evidence that acetyl-CoA availability correlates with histone acetylation came from studies in *Saccharomyces cerevisiae*. Yeast cells grown in a chemostat with limited glucose supply oscillate synchronously between three distinctive metabolic phases in a so-called 'yeast metabolic cycle' (YMC) (Figure 3B) [68,69]. The first oxidative phase (OX) is characterized by high oxygen consumption, accumulation of building blocks, sulphur metabolism, as well as ribosome and amino acid synthesis. Following, in the reductive-building phase (RB), genes involved in mitochondria biogenesis and responsible for DNA replication and cell division show the highest expression. The last phase, reductive-charging (RC), is characterized by intensive fatty acid oxidation and glycolysis [70]. Oscillations in acetyl-CoA concentration follow the periodicity of YMC and peak at the transition from OX to RB phase. Notably, acetylation levels of multiple histone Lys residues (including K9, K14, K23, and K27 on H3 and K5, K8, and K12 on H4) are tightly correlated with these changes in acetyl-CoA levels (Figure 3C). Moreover adding acetate, ethanol, acetaldehyde, and lactate during a reductive phase forces yeast cells to rapidly enter the oxidative phase, which is mirrored by acquiring additional acetylation marks on histones [68]. This clearly demonstrates that, in yeast, acetyl-CoA availability, which reflects the metabolic state of the cell, is a crucial factor regulating histone acetylation levels. In mammals, acetyl-CoA levels do not change in a regular manner but can vary depending on the stage of development. Its abundance in mESCs is about 8 times higher than in embryoid bodies induced by withdrawal of LIF and application of retinoic acid for 7 days. Elevated levels of acetyl-CoA is caused by increased Tdh expression in mESCs but whether this has an impact on histone acetylation has not been investigated [36]. In many cancer-derived cell lines, high levels of glucose in the medium result in increased amounts of acetyl-CoA and are accompanied by an elevation in global histone acetylation levels compared to non-cancer cells. Notably, the exact effect of glucose supplementation on modification of specific histone Lys residues varies between cell lines [71]. This observation, that in many different cellular models the availability of acetyl-CoA determines the level of histone acetylation, implies that HATs are substrate limited enzymes that indeed respond to alterations in the energetic state of the cell.

6. NAD$^+$ AS A COFACTOR OF SIRTUINS

6.1. NAD$^+$ dependent histone deacetylation

The oxidized form of nicotinamide adenine dinucleotide (NAD$^+$) is an important redox cofactor involved in catabolic and oxidative pathways that accepts electrons in dehydrogenase reactions in the TCA cycle, glycolysis and β-oxidation. Electrons from its reduced form, NADH, are constantly removed and used in the mitochondrial electron transport chain, resulting in oxidative phosphorylation and ATP generation. Apart from its well established function in aerobic respiration, NAD$^+$ also plays a role in calcium mobilization, thus in signaling pathways, ADP-ribosylation, and protein deacetylation [72–74]. Histone deacetylases (HDACs) are enzymes removing acetyl groups from histones and non-histone proteins. So far, 18 HDACs have been identified in humans and are grouped into four classes. The classical HDACs from class I, II, and IV share a catalytic mechanism that requires a zinc ion and does not depend on cellular metabolism-derived compounds; therefore, discussing these HDACs would be beyond the scope of this article. HDACs from class III, named sirtuins after their homologue yeast protein Sir2, are the only class that require NAD$^+$ as a cofactor; therefore, they are the prime candidates to be metabolic sensors of NAD$^+$/NADH fluctuations that couple the energetic state of the cell with histone acetylation levels and gene expression [75,76]. Early work in sirtuin studies showed that in *S. cerevisiae* NAD$^+$-dependent histone deacetylation by Sir2 is required for silencing at mating-type loci Hidden MAT Left (HML) and Hidden MAT Right (HMR), at telomeres and rDNA locus RDN1 [77]. Even though no direct link between the levels of NAD$^+$ and silencing of these loci has been reported so far, the fact that NAD$^+$ is required for particular changes in chromatin structure supports the concept of NAD$^+$ as one of the hubs of metabolism-to-chromatin signaling.

Sirtuins are conserved in all species from bacteria, in which usually 1 or 2 sirtuins are present, to higher eukaryotes encoding multiple versions of them. In mammals, there are 7 sirtuins: four nucleocytosolic (SIRT1, SIRT2, SIRT6, and SIRT7) and three mitochondrial (SIRT3, SIRT4, SIRT5) [78–80]. SIRT activity is inhibited by several exogenous small molecules (splitomicin, sirtinol, and its analogues, EX-527) and also by NADH and NAM whose availability in mammals is tightly coupled to NAD$^+$ metabolism [79,81], providing a particularly relevant link with metabolism.

6.2. NAD$^+$ metabolism

In mammals, there are two pathways in which NAD$^+$ is generated (Figure 4). The *de novo* pathway involves several steps of tryptophan conversion and is rate limited by the enzyme catalyzing the first step, indoleamine-pyrrole 2,3-dioxygenase (IDO). In a salvage (recycling) pathway, nicotinamide (NAM) generated by NAD$^+$-consuming enzymes can be converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT), the rate limiting enzyme in this salvage pathway, and further to NAD$^+$ by nicotinamide mononucleotide adenylyltransferase (NMNAT) [82]. Upon inhibition of NAMPT the lifespan of smooth muscle cells, previously shown to be regulated by SIRT1, was reduced due to decreased NAD$^+$ production [83]. This indicates that the turnover of NAD$^+$ by NAMPT is important for proper SIRT1 function. There are three isoforms of NMNAT: NMNAT1, found mostly in the nucleus, has been reported to interact with SIRT1 in a complex present in chromatin and affecting histone acetylation [84], whereas the roles of NMNAT2 (the cytosolic isoform) and NMNAT3 (the mitochondrial isoform) in histone acetylation state have not been investigated [85]. While the mitochondrial pool of NAD$^+$ is preferentially used for ATP generation, its function in the nucleus is not that explicit. Apart from its role in histone deacetylation, NAD$^+$ is also used by poly(ADP-ribose) polymerases (PARP) acting in close proximity to DNA and histones as well as by cyclic ADP-ribose synthases, enzymes mainly localized on the cell surface but also present in the nuclear inner envelope [86]. Therefore, the interaction of NMNAT with particular chromatin complexes can be a mechanism favoring one of the competing nuclear pathways.

6.3. NAD$^+$ levels sensed by nuclear enzymes

The circadian clock controls the rhythmic expression of many eukaryotic genes and modulates various physiological functions by feedback loops that involve a set of transcriptional factors and their regulators. In mammals, the major regulators of circadian rhythms are the heterodimeric transcription factors CLOCK and BMAL1 that activate the expression of their transcriptional repressors Period (*Per1*, *Per2*) and Cryptochrome (*Cry1*, *Cry2*) [87]. The core circadian clock machinery BMAL1:CLOCK controls, among others, the expression of

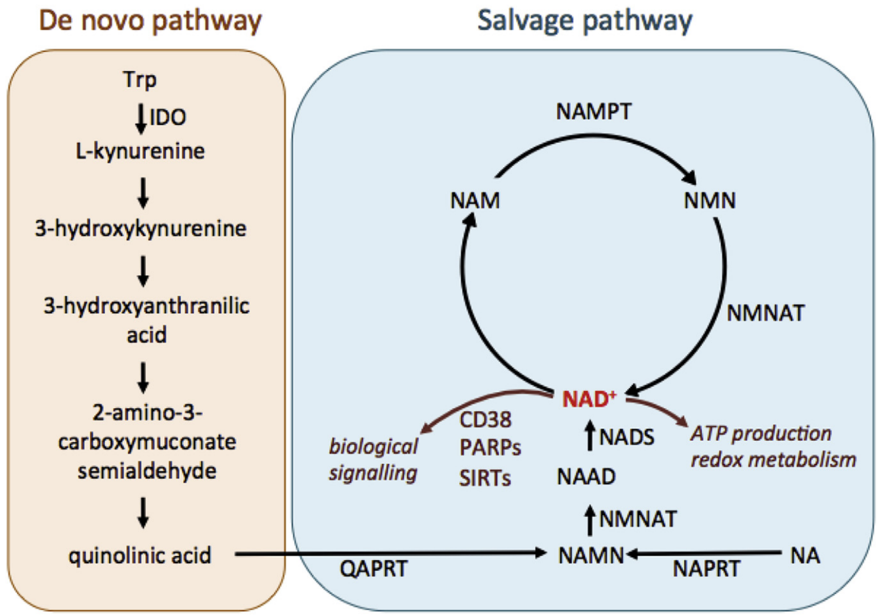

Figure 4: Nicotinamide Adenine Dinucleotide (NAD^+) synthesis pathways. *De novo* synthesis NAD^+ from tryptophan (Trp) occurs mainly in the liver. The 'salvage' generation of NAD^+ can result from either nicotinic acid (NA) or from a recycling pathway. Recycling of nicotinamide (NAM), the by-product of NAD^+-dependent enzymes' activity, is driven by nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide adenylyltransferase (NMNAT) with nicotinamide mononucleotide (NMN) as an intermediary product; CD38 — cyclic ADP-ribose hydrolase 1; IDO — indoleamine 2,3-dioxygenase; NAAD - nicotinic acid adenine dinucleotide; NADS — nicotinamide adenine dinucleotide synthetase; NAMN — nicotinic acid mononucleotide; NAPRT — nicotinic acid phosphoribosyltransferase; PARPs — poly(ADP-ribose) polymerase; QAPRT — quinolinate phosphoribosyltransferase; SIRTs — sirtuins.

NAMPT. Although expression of sirtuins is constant, their HDAC activity on H3 depends on the level of NAMPT and therefore oscillates in a circadian manner mirroring the circadian fluctuations of NAD^+ [88]. In *Clock* knockout mice, upon inhibition of NAMPT, both circadian oscillations of NAD^+ and SIRT1 activity are largely lost [8]. Thus, SIRT1 activity seems to be regulated by circadian fluctuations of NAD^+. Whether alterations of NAD^+ levels caused by starvation or calorie restriction contribute significantly to sirtuins' activity is still controversial. It is commonly accepted that even under changed metabolic conditions the NAD^+ level is kept largely constant. However, it should be noted that what can affect sirtuins' activity is the $[NAD^+]$:[NADH] ratio. Studies in skeletal myoblasts showed that upon reducing glucose concentration in culture medium from 25 mM to 5 mM, the $[NAD^+]$:[NADH] ratio increased from 5 to 15 [89]. Moreover, measuring total cellular concentration of NAD^+ can mask slight but important changes within subcellular compartments. A genetically encoded fluorescent biosensor, an excellent tool to measure intracellular concentrations of metabolites, determined the concentration of NAD^+ in HEK293T nucleus to be 109 μM [90]. Interestingly, this value is very close to the previously determined SIRT1 dissociation constant for NAD^+ ($K_m = 94$ μM) [91]. Therefore, it is plausible that subtle alterations in the nuclear concentrations of NAD^+ and its inhibitors, mainly NADH and NAM, can be sensed by SIRT1 and thus, couple energetic state with histone deacetylation.

As mentioned before, there are two predominant groups of nuclear NAD^+ dependent enzymes: class III HDACs and PARPs. The majority of PARP activity is distributed between PARP-1 and PARP-2 [92]. As the K_m of PARP-1 for NAD^+ is below its nuclear concentration it is unlikely that the activity of PARP-1 is majorly modulated by fluctuations of NAD^+ [93]. However, PARP-1 activity can reduce the effective concentration of NAD^+ available for other enzymes. It has been shown that the consumption of NAD^+ by constitutive activation of PARP-1 decreases SIRT1 activity and causes dysregulation of SIRT1 target genes in Xeroderma pigmentosum and Cockayne syndrome [94,95]. The PARP-2 dissociation constant for NAD^+ is within the range of physiological changes in NAD^+ concentration ($K_m = 130$ μM) and thus PARP-2 can directly compete with SIRT1 for the 'shared' cofactor.

Taken together, there are many lines of evidence indicating that NAD^+ is a powerful signaling molecule transferring information about the energetic status of the cell to the chromatin level. However, precise measurements of free NAD^+ and NADH in subcellular compartments are still challenging. Further studies are also required to investigate both the nuclear-wide and gene-specific effects of varying NAD^+ concentration, as well as potential effects on 'chromatin microdomains'.

7. SHORT CHAIN ACYL-COAS AS COFACTORS OF HISTONE ACYLATIONS

Owing to the advantages of high-sensitivity mass spectrometry, several new short-chain Lys acylations have recently been discovered on histones: propionylation, butyrylation, crotonylation, 2-hydroxyisobutyrylation, succinylation, malonylation, glutarylation, and β-hydroxybutyrylation [96–102], and their roles in transcriptional regulation are currently under careful investigation. Of note, the vast majority of novel modifications were identified on Lys residues that can also be acetylated. Different chemical properties of the various acyl groups (Table 1) could allow various 'reader' proteins to interact preferentially with specifically acylated Lys residues and thus add more complexity to the previously established 'histone code' hypothesis [103]. So far, specific 'writers' for distinct non-acetyl acylation have not been identified, but rather several studies showed that HATs from all three families can catalyze different acylations albeit with reduced kinetics. A promising candidate for a short chain acyltransferase is p300 that, due to the presence of a deep aliphatic pocket in its active

Table 1 — Overview of different histone acylations and their functions in transcription; AA — amino acids, AKI — acute kidney injury, BCAA — branched-chain amino acids, MSCI — meiotic sex chromosome inactivation, SCFA — short-chain fatty acids.

Type of acylation	Chemical properties of acyl group	Modification site on histones (mouse)	Source of coAs	Function	References
Propionylation	Hydrophobic	Mainly N-terminal tails of H3 and H4	SCFA oxidation, BCAA catabolism	Gene activation	[109]
Butyrylation	Hydrophobic	Mainly N-terminal tails of H3 and H4, globular domains of H3 and H4	SCFA oxidation, BCAA catabolism	Gene activation, role in spermatogenesis	[107]
2-Hydroxyisobutyrylation	Polar	Both N-terminal tails and globular domains	BCAA catabolism	Sustaining transcription of genes escaping MSCI in spermatogenesis	[100]
Succinylation	Acidic	Mainly globular domains of H3, H4, H1	SCFA oxidation	Unknown	
Malonylation	Acidic	Mainly globular domains of H3, H4, H1	Lipogenesis	Unknown	
Glutarylation	Acidic	Mainly C-terminal tail of H2B	AA catabolism	Unknown	
Crotonylation	Hydrophobic	Both N-terminal tails and globular domains	SCFA oxidation	Signal dependent gene activation, sustaining transcription of genes escaping MSCI in spermatogenesis, role in AKI-stress induced gene activation	[66,97,102]
β-Hydroxybutyrylation	Polar	Both N-terminal teils and globular domains	SCFA oxidation, ketogenesis	Starvation induced gene activation	[106]

site, can accommodate substrates with increased acyl-chain length like propionyl-, butyryl- and crotonyl-CoA [104]. Therefore the patterns of histone Lys acylations could be established by competition of different acyl-CoAs. Under this assumption, relative concentrations of nuclear acyl-CoAs, generated in many metabolic pathways (Table 1), could be an important player in sensing the metabolic state of a cell and transferring this information to chromatin via distinct histone acylations.

Short chain acyl-CoAs are metabolites involved in various anabolic and catabolic pathways, the regulation of which depends on the energetic state of the cell. When glucose sources are limited (e.g. upon fasting or reduced intake of carbohydrates), ketone bodies (acetoacetate, β-hydroxybutyrate [βOHB], and, to a lesser extent, acetone) are produced by the liver, secreted, and serve as an alternative energy source in extrahepatic tissues. In heart, brain, or muscles, βOHB can be converted into acetyl-CoA to fuel the TCA cycle or, alternatively, it can be used to generate βOHB-CoA — a cofactor for Lys β-hydroxybutyrylation (bhb). Structural similarity of βOHB to the well-known HDAC inhibitor butyrate implied its potential role in deacetylation inhibition, which was confirmed by an increase in H3K9 and H3K14 acetylation levels upon treatment of HEK293 cells with increasing amounts of βOHB [105]. In another study, however, starvation of mice or treatment of cells with exogenous βOHB led to a 10-fold—40-fold increase in histone Kbhb levels whereas histone acetylation levels were not dramatically changed [106]. These two observations suggest a dual role for βOHB in the regulation of histone acylation states — as both an HDAC inhibitor and a cofactor for β-hydroxybutyrylation. Interestingly, increase in H3K9bhb upon starvation was positively correlated with active gene expression [106]. Since its first identification, histone Kbhb has been found on many Lys residues within all histones.

Histone Lys propionylation (pr), butyrylation (bu), and crotonylation (cr) have also been linked with active transcription [66,96,107]. Butyrate is naturally produced by the gastrointestinal microbiota or orally ingested as a feed additive and serves as an important energy source for colonocytes that use it as a substrate for β-oxidation and production of acetyl-CoA [108]. While excess butyrate can be transported to the liver, so far, the effect of butyrate has been explored mainly in colonocytes where its inhibitory effect on class I and IIa HDACs favors histone acetylation, and here it contributes to increased concentration of acetyl-CoA via intensified β-oxidation. These mechanisms together with increased H4 butyrylation could promote active transcription. H3K14pr and H3K14bu are also novel acylation marks found alongside H3K9ac within the promoters of active genes in mouse livers [109], where the enrichment profile of H3K14pr is strongly correlated with transcriptional activity. Both of these H3K14 acylations could serve as elements of a regulatory feedback mechanism signaling a certain metabolic state to chromatin. In line with this, in a cell-free *in vitro* transcription system, propionyl-CoA stimulates transcription to a comparable extent as acetyl-CoA implying that 'activation potential' of propionylated and acetylated histones is similar.

Apart from deacetylation, some sirtuins also show short chain deacylation activity. Human SIRT1 can depropionylate H3K23 *in vitro*, although the efficiency is lower than on an acetylated peptide [110]. Interestingly, mitochondrial SIRT5 displays higher demalonylation and desuccinylation activity than deacetylation both *in vitro* and *in vivo* [111] due to the presence of arginine and tyrosine residues in the acyl pocket of SIRT5 that favor the accommodation of succinyl and malonyl group. Therefore, careful structural analysis of active sites of nuclear sirtuins could shed more light on their potential 'additional' activities regulating histone short chain acylations.

8. OUTLOOK

As described in this review, there is growing evidence that various chromatin-modifying enzymes can sense and respond to changes in the levels of metabolites that are their cofactors, cosubstrates, or inhibitors. The fact that many histone acetyltransferases, histone methyltransferases, DNA methyltransferases, and their corresponding 'erasers' can be (in contrast to kinases whose K_m for ATP is significantly lower than its cellular concentration) substrate limited suggests links between the metabolic condition of a cell, chromatin architecture, and, hence, gene expression programs [112,113]. However, there are many questions that remain open, including whether the altered levels of metabolites have a global effect on chromatin or if specific genes respond preferentially to these changes. The availability of genetically encoded sensors providing localized measurements of metabolite concentrations *in vivo* in subcellular compartments could help to address this question and allow potential chromatin microdomains,

Table 2 — Kinetic parameters and cellular concentrations of cofactors used by chromatin-modifying enzymes; CoA — coenzyme A, FAD/FADH2 — oxidized/reduced form of flavin adenine dinucleotide, MTA — 5′-methylthioadenosine, NAD^+/NADH — oxidized/reduced form of nicotinamide adenine dinucleotide, NAM — nicotinamide, R-2HG - R enantiomer of 2-hydroxyglutarate, SAH — S-adenosylhomocysteine, SAM — S-adenosylmethionine, α-KG — α-ketoglutarate, 2-OGDO — Fe^{2+} and α-KG dependent dioxygenases;* - histone acetyltransferases with reported acyltransferase activity: p300, MOF, PCAF, GCN5, TIP60.

Enzyme	Cofactors (endogenous metabolites)	Inhibitors (endogenous metabolites)	K_m for cofactor [μM]	Cellular/nuclear concentration of cofactor [μM]	Localisation of cofactor production	References
Histone acetyltransferases	Acetyl-CoA	CoA, acyl-CoAs	0.2—46	2—20/?	Cytosol, mitochondria, nucleus	[71,101,117] [118,119]
Histone methyltransferases	SAM	SAH, MTA	1.2—34.5	3.3—59/?	Cytosol, nucleus	[120—122]
DNA methyltransferases	SAM	SAH, MTA	0.1—21	3.3—59/?	Cytosol, nucleus	[122,123] [124—126]
Histone acyltransferases*	acyl-CoA	CoA		0.1—1.5/?	Cytosol, nucleus	[101]
Sirtuins	NAD^+	NAM, NADH	2.3—1400	300—2000/ 87—136	Cytosol, mitochondria, nucleus	[79,91,127,128] [129—131]
Histone demethylases, 2-OGDO	α-KG	R-2HG, succinate, fumarate	9—37	110—260/?	Cytosol, mitochondria	[53,132—135]
Lsd1/2	FAD	$FADH_2$	?	?/?	Cytosol, mitochondria	
DNA demethylation	α-KG	R-2HG, succinate, fumarate	35—75	110—260/?	Cytosol, mitochondria	[135—138]

discriminated by altered local concentrations of specific metabolites, to be studied. Changes in e.g. the subnuclear distribution of particular compounds could thus have an impact on chromatin modifications even if their total cellular concentrations remain constant. Many of the metabolites described in this article are of particular interest since various studies, summarized in Table 2 have reported their cellular concentrations to be within the range of dissociation constants of the enzymes using them as cofactors. However, due to the facts that: (i) certain cellular metabolites can serve also as inhibitors of these enzymes and (ii) kinetic parameters are mainly measured using *in vitro* methods, obtained values may not fully reflect enzymes' properties *in vivo*. Further studies focused on enzymes' kinetics and distribution of metabolites in intracellular compartments are necessary and constitute an interesting direction for future research.

Furthermore, as many of these key cellular metabolites are involved in a plethora of pathways, translocation of the enzymes synthesising them to the nucleus could be an important regulator of metabolic-chromatin crosstalk. Interestingly, components of the pyruvate dehydrogenase complex (PDC) and α-ketoglutarate dehydrogenase (α-KGDH) complex, which have previously been found mainly in mitochondria, have recently been discovered in the nucleus [67,114]. Therefore, the possibility that under certain conditions more enzymes whose primary function was linked to other subcellular compartments could be translocated to the nucleus, cannot be excluded. Further investigation is needed to define signals triggering such translocations and mechanisms involved in this processes.

Remarkably, there is also a clear disease relevance of the coupling between the metabolic state and chromatin modifications. Multiple connections between impaired activity of some TCA cycle enzymes and various human diseases have been discovered in the last few years. Loss- and gain-of-function mutations of *SDH*, *FH*, and *IDH1/2*, respectively, have been associated with cancer phenotypes [53,56]. Although these mutations correlated in *in vitro* cellular models with changes in histone and DNA methylation levels, the exact molecular mechanisms in cancer tissues, and thus potential therapeutic approaches, are still unknown.

Beyond the usual suspects, novel modifications of DNA and RNA and their first links with metabolism have also recently been reported. Fat mass and obesity-associated protein (FTO) has been shown to affect human obesity and energy homeostasis [115]. Interestingly, FTO is a demethylase with the highest activity towards m^6A, the most abundant RNA modification in mammals. This indicates that at least some of the many RNA modifications could also be linked to metabolism [116]. However, this field of novel DNA/RNA modifications is still in its infancy. We are getting more and more evidence of the threads linking epigenetic modifications and metabolism. Uncovering the dynamic relationship between various chromatin marks and the metabolic state of the cell shaped by multiple factors is still challenging and metaboloepigenetic studies that comprehensively investigate all the epigenetic changes (e.g. DNA, RNA and histone modifications, chromatin architecture) caused by specific alterations of metabolic state (e.g. by distribution and activity of metabolic enzymes and metabolites) would be of great importance for the community. Adding to the challenge is the fact that new players in the form of modifications, 'writers', and 'erasers' are constantly being identified. Nevertheless we anticipate that the growing interest in the emerging field of metaboloepigenetics will lead us to a better understanding of these reciprocal links.

CONFLICT OF INTEREST

None.

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REFERENCES

[1] Waddington, C.H., 1942. The epigenotype. Endeavour, 18—20.
[2] Berger, S.L., Kouzarides, T., Shiekhattar, R., Shilatifard, A., 2009. An operational definition of epigenetics an operational definition of epigenetics. Genes & Development, 781—783.
[3] Feil, R., Fraga, M.F., 2012. Epigenetics and the environment: emerging patterns and implications. Nature Reviews Genetics 13(2):97—109.
[4] Kim, Y.-I., 2005. Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. Journal of Nutrition 135(11): 2703—2709.
[5] Lu, C., Thompson, C.B., 2012. Metabolic regulation of epigenetics. Cell Metabolism 16(1):9—17.

[6] Katada, S., Imhof, A., Sassone-Corsi, P., 2012. Connecting threads: epigenetics and metabolism. Cell 148(1–2):24–28.

[7] Kaelin, W.G., McKnight, S.L., 2013. Influence of metabolism on epigenetics and disease. Cell 153(1):56–69.

[8] Gut, P., Verdin, E., 2013. The nexus of chromatin regulation and intermediary metabolism. Nature 502(7472):489–498.

[9] Janke, R., Dodson, A.E., Rine, J., 2015. Metabolism and epigenetics. Annual Review of Cell and Developmental Biology 31:473–496.

[10] J. A. Van Der Knaap and C. P. Verrijzer, "Undercover : gene control by metabolites and metabolic enzymes,"Genes & Development pp. 2345–2369.

[11] Reid, M.A., Dai, Z., Locasale, J.W., 2017. The impact of cellular metabolism on chromatin dynamics and epigenetics. Nature Cell Biology 19(11): 1298–1306.

[12] Lyon, M.F., 1961. Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature 192:372–373.

[13] Bacolla, A., Pradhan, S., Roberts, R.J., Wells, R.D., 1999. Recombinant human DNA (Cytosine-5) methyltransferase. Journal of Biological Chemistry 274(46):33002–33010.

[14] Kossel, A., 1884. Uber einen peptonartigen bestandteil des zellkerns. Zeitschrift fuer Physiologische Chemie(5):511–515.

[15] Allfrey, V.G., Faulkner, R., Mirsky, A.E., 1964. Acetylation and methylation of histones and their possible role in the regulation of Rna synthesis. Proceedings of the National Academy of Sciences of the United States of America 51(1938):786–794.

[16] Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D., Klug, A., 1984. Structure of the nucleosome core particle at 7 resolution. Nature 311(5986):532–537.

[17] Rossetto, D., Avvakumov, N., Côté, J., 2012. Histone phosphorylation: a chromatin modification involved in diverse nuclear events. Epigenetics 7(10): 1098–1108.

[18] Greer, E.L., Shi, Y., 2012. Histone methylation: a dynamic mark in health, disease and inheritance. Nature Reviews Genetics 13(5):343–357.

[19] Shahbazian, M.D., Grunstein, M., 2007. Functions of site-specific histone acetylation and deacetylation. Annual Review of Biochemistry 76(1): 75–100.

[20] Weake, V.M., Workman, J.L., 2008. Histone ubiquitination: triggering gene activity. Molecular Cell 29(6):653–663.

[21] Huisinga, K.L., Pugh, B.F., 2004. A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. Molecular Cell 13(4):573–585.

[22] Margueron, R., Justin, N., Ohno, K., Sharpe, M.L., Son, J., Iii, W.J.D., et al., 2009. Role of the polycomb protein Eed in the propagation of repressive histone marks. Nature 461(7265):762–767.

[23] Di Cerbo, V., Mohn, F., Ryan, D.P., Montellier, E., Kacem, S., Tropberger, P., et al., 2014. Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription. Elife 3:1–23.

[24] Iwasaki, W., Tachiwana, H., Kawaguchi, K., Shibata, T., Kagawa, W., Kurumizaka, H., 2011. Comprehensive structural analysis of mutant nucleosomes containing lysine to glutamine (KQ) substitutions in the H3 and H4 histone-fold domains. Biochemistry 50(36):7822–7832.

[25] Tropberger, P., Pott, S., Keller, C., Kamieniarz-Gdula, K., Caron, M., Richter, F., et al., 2013. Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. Cell 152(4):859–872.

[26] Bradley, R.S., Hughes, M.K., Crowley, T.J., Baum, S.K., Kim, K.Y., Hyde, W.T., et al., 2006. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science (80-.)(February):844–848.

[27] Gold, M., Hurwitz, J., Anders, M., 1963. The enzymatic methylation of RNA and DNA. Biochemical and Biophysical Research Communications 11(2): 107–114.

[28] Feinberg, A.P., Vogelstein, B., 1983. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301(5895): 89–92.

[29] Zhang, W., Xu, J., 2017. DNA methyltransferases and their roles in tumorigenesis. Biomaterials Research 5(1):1.

[30] Kaneda, M., Okano, M., Hata, K., Sado, T., Tsujimoto, N., Li, E., et al., 2004. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature 429(6994):900–903.

[31] Hermann, A., Goyal, R., Jeltsch, A., 2004. The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. Journal of Biological Chemistry 279(46): 48350–48359.

[32] Wei, C.M., Gershowitz, A., Moss, B., 1975. Methylated nucleotides block 5′ terminus of HeLa cell messenger RNA. Cell 4(4):379–386.

[33] Cui, Q., Shi, H., Ye, P., Li, L., Qu, Q., Sun, G., et al., 2017. m6A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. Cell Reports 18(11):2622–2634.

[34] Day, C.R., Kempson, S.A., 2016. Betaine chemistry, roles, and potential use in liver disease. Biochimica et Biophysica Acta (BBA) – General Subjects 1860(6):1098–1106.

[35] Wolff, G.L., Kodell, R.L., Moore, S.R., Cooney, C.A., 1998. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. The FASEB Journal 12(11):949–957.

[36] Wang, J., Alexander, P., Wu, L., Hammer, R., Cleaver, O., McKnight, S.L., 2009. Dependence of mouse embryonic stem cells on threonine catabolism. Science (80-.). 325(5939):435–439.

[37] Shyh-Chang, N., Locasale, J.W., Lyssiotis, C.A., Zheng, Y., Teo, R.Y., Ratanasirintrawoot, S., et al., 2013. Influence of threonine metabolism on S-Adenosylmethionine and histone methylation. Science (80-.). 339(6116): 222–226.

[38] Edgar, A.J., 2002. The human L-threonine 3-dehydrogenase gene is an expressed pseudogene. BMC Genetics 3:18.

[39] Mentch, S.J., Mehrmohamadi, M., Huang, L., Liu, X., Gupta, D., Mattocks, D., et al., 2015. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. Cell Metabolism 22(5): 861–873.

[40] Kera, Y., Katoh, Y., Ohta, M., Matsumoto, M., Takano-Yamamoto, T., Igarashi, K., 2013. Methionine adenosyltransferase II-dependent histone H3K9 methylation at the COX-2 gene locus. Journal of Biological Chemistry 288(19):13592–13601.

[41] Ding, W., Smulan, L.J., Hou, N.S., Taubert, S., Watts, J.L., Walker, A.K., 2015. S-adenosylmethionine levels govern innate immunity through distinct methylation-dependent pathways. Cell Metabolism 22(4):633–645.

[42] Towbin, B.D., González-Aguilera, C., Sack, R., Gaidatzis, D., Kalck, V., Meister, P., et al., 2012. Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. Cell 150(5):934–947.

[43] Li, S., Swanson, S.K., Gogol, M., Florens, L., Washburn, M.P., Jerry, L., et al., 2015. Serine and SAM responsive complex SESAME regulates histone modification crosstalk by sensing cellular metabolism. Mol. Cell 60(3): 408–421.

[44] Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., et al., 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 119(7):941–953.

[45] Karytinos, A., Forneris, F., Profumo, A., Ciossani, G., Battaglioli, E., Binda, C., et al., 2009. A novel mammalian flavin-dependent histone demethylase. Journal of Biological Chemistry 284(26):17775–17782.

[46] Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., et al., 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science (80-.). 324(5929):930–935.

[47] Kohli, R.M., Zhang, Y., 2013. TET enzymes, TDG and the dynamics of DNA demethylation. Nature 502(7472):472–479.

[48] Hahn, M.A., Qiu, R., Wu, X., Li, A.X., Zhang, H., Wang, J., et al., 2013. Dynamics of 5-hydroxymethylcytosine and chromatin marks in mammalian neurogenesis. Cell Reports 3(2):291–300.

[49] Wu, N., Yang, M., Gaur, U., Xu, H., Yao, Y., Li, D., 2016. Alpha-ketoglutarate: physiological functions and applications. Biological Therapy (Seoul) 24(1): 1–8.

[50] Carey, B.W., Finley, L.W.S., Cross, J.R., Allis, C.D., Thompson, C.B., 2014. Intracellular α-ketoglutarate maintains the pluripotency of embryonic stem cells. Nature.

[51] Salminen, A., Kauppinen, A., Kaarniranta, K., 2015. 2-Oxoglutarate-dependent dioxygenases are sensors of energy metabolism, oxygen availability, and iron homeostasis: potential role in the regulation of aging process. Cellular and Molecular Life Sciences 72(20):3897–3914.

[52] Pollard, P.J., Brière, J.J., Alam, N.A., Barwell, J., Barclay, E., Wortham, N.C., et al., 2005. Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. Human Molecular Genetics 14(15):2231–2239.

[53] Xiao, M., Yang, H., Xu, W., Ma, S., Lin, H., Zhu, H., et al., 2012. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes & Development 26(12):1326–1338.

[54] Al-Khallaf, H., 2017. Isocitrate dehydrogenases in physiology and cancer: biochemical and molecular insight. Cell & Bioscience 7(1):37.

[55] Reitman, Z.J., Parsons, D.W., Yan, H., 2010. IDH1 and IDH2: not your typical oncogenes. Cancer Cell 17(3):215–216.

[56] Figueroa, M.E., Abdel-Wahab, O., Lu, C., Ward, P.S., Patel, J., Shih, A., et al., 2010. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 18(6):553–567.

[57] Sabari, B.R., Zhang, D., Allis, C.D., Zhao, Y., 2016. Metabolic regulation of gene expression through histone acylations. Nature Reviews Molecular Cell Biology 18(2):90–101.

[58] Thorne, A.W., Kmiciek, D., MitchelsonN, K., Sautiere, P., Crane-Robinson, C., 1990. Patterns of histone acetylation. European Journal of Biochemistry 193(3):701–713.

[59] Pradeepa, M.M., Grimes, G.R., Kumar, Y., Olley, G., Taylor, G.C.A., Schneider, R., et al., 2016. Histone H3 globular domain acetylation identifies a new class of enhancers. Nature Genetics 48(April):681–686.

[60] Hassan, A.H., Prochasson, P., Neely, K.E., Galasinski, S.C., Chandy, M., Carrozza, M.J., et al., 2002. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell 111(3):369–379.

[61] Filippakopoulos, P., Knapp, S., 2012. The bromodomain interaction module. FEBS Letters 586(17):2692–2704.

[62] Filippakopoulos, P., Knapp, S., 2014. Targeting bromodomains: epigenetic readers of lysine acetylation. Nature Reviews Drug Discovery 13(5): 337–356.

[63] Albaugh, B.N., Arnold, K.M., Denu, J.M., 2011. KAT(ching) metabolism by the tail: insight into the links between lysine acetyltransferases and metabolism. ChemBioChem 12(2):290–298.

[64] Mews, P., Donahue, G., Drake, A.M., Luczak, V., Abel, T., Berger, S.L., 2017. Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. Nature 546(7658):381–386.

[65] Wellen, K.E., Hatzivassiliou, G., Sachdeva, U.M., V Bui, T., Cross, J.R., Thompson, C.B., 2009. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324(5930):1076–1080.

[66] Sabari, B.R., Tang, Z., Huang, H., Yong-Gonzalez, V., Molina, H., Kong, H.E., et al., 2015. Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. Mol. Cell 58(2):203–215.

[67] Sutendra, G., Kinnaird, A., Dromparis, P., Paulin, R., Stenson, T.H., Haromy, A., et al., 2014. A nuclear pyruvate dehydrogenase complex is important for the generation of Acetyl-CoA and histone acetylation. Cell 158(1):84–97.

[68] Cai, L., Sutter, B.M., Li, B., Tu, B.P., 2011. Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell 42(4):426–437.

[69] Tu, B.P., Mohler, R.E., Liu, J.C., Dombek, K.M., Young, E.T., Synovec, R.E., et al., 2007. Cyclic changes in metabolic state during the life of a yeast cell. Proceedings of the National Academy of Sciences of the United States of America 104(43):16886–16891.

[70] Tu, B.P., 2005. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science (80-.) 310(5751):1152–1158.

[71] Lee, J.V., Carrer, A., Shah, S., Snyder, N.W., Wei, S., Venneti, S., et al., 2014. Akt-dependent metabolic reprogramming regulates tumor cell Histone acetylation. Cell Metabolism 20(2):306–319.

[72] Guse, A.H., 2015. Calcium mobilizing second messengers derived from NAD. Biochimica et Biophysica Acta 1854(9):1132–1137.

[73] Schreiber, V., Dantzer, F., Ame, J.-C., de Murcia, G., 2006. Poly(ADP-ribose): novel functions for an old molecule. Nature Reviews Molecular Cell Biology 7(7):517–528.

[74] Imai, S., Armstrong, C.M., Kaeberlein, M., Guarente, L., 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403(6771):795–800.

[75] Rine, J., Herskowitz, I., 1987. Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. Genetics 116(1):9–22.

[76] Guarente, L., 2000. Sir2 links chromatin silencing, metabolism, and aging. Genes & Development 14(9):1021–1026.

[77] Smith, J.S., Brachmann, C.B., Pillus, L., Boeke, J.D., 1998. Distribution of a limited Sir2 protein pool regulates the strength of yeast rDNA silencing and is modulated by Sir4p. Genetics 149(3):1205–1219.

[78] Frye, R.A., 2000. Phylogenetic classification of prokaryotic and eukaryotic sir2-like proteins. Biochemical and Biophysical Research Communications 273(2):793–798.

[79] Haigis, M.C., Sinclair, D.A., 2010. Mammalian sirtuins: biological insights and disease relevance. Annual Review of Pathology: Mechanisms of Disease 5(1): 253–295.

[80] Rack, J.G.M., Vanlinden, M.R., Lutter, T., Aasland, R., Ziegler, M., 2014. Constitutive nuclear localization of an alternatively spliced sirtuin-2 isoform. Journal of Molecular Biology 426(8):1677–1691.

[81] Bitterman, K.J., Anderson, R.M., Cohen, H.Y., Latorre-Esteves, M., Sinclair, D.A., 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2 and human SIRT1. Journal of Biological Chemistry 277(47):45099–45107.

[82] Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., Sassone-Corsi, P., 2009. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science (80-.). 324(5927):654–657.

[83] Van Der Veer, E., Ho, C., O'Neil, C., Barbosa, N., Scott, R., Cregan, S.P., et al., 2007. Extension of human cell lifespan by nicotinamide phosphoribosyltransferase. Journal of Biological Chemistry 282(15):10841–10845.

[84] Zhang, T., Kraus, W.L., 2010. SIRT1-dependent regulation of chromatin and transcription: linking NAD+ metabolism and signaling to the control of cellular functions. Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics 1804(8):1666–1675.

[85] Ali, Y.O., Li-Kroeger, D., Bellen, H.J., Zhai, R.G., Lu, H.C., 2013. NMNATs, evolutionarily conserved neuronal maintenance factors. Trends in Neurosciences 36(11):632–640.

[86] Khoo, Keng Meng, Han, M.K., Park, J.B., Chae, Soo Wan, Kim, U.H., Lee, Hon Cheung, et al., 2000. Localization of the cyclic ADP-ribose-dependent calcium signaling pathway in hepatocyte nucleus. Journal of Biological Chemistry 275(32):24807–24817.

[87] Ko, C.H., Takahashi, J.S., 2006. Molecular components of the mammalian circadian clock. Human Molecular Genetics 15(2):271–277.

[88] Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., et al., 2008. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134(2):329–340.
[89] Fulco, M., Cen, Y., Zhao, P., Hoffman, E.P., McBurney, M.W., Sauve, A.A., et al., 2008. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of nampt. Developmental Cell 14(5):661–673.
[90] Cambronne, X.A., Stewart, M.L., Kim, D., Jones-Brunette, A.M., Morgan, R.K., Farrens, D.L., et al., 2016. Biosensor reveals multiple sources for mitochondrial NAD+. Science (80-.) 352(6292):1474–1477.
[91] Pacholec, M., Bleasdale, J.E., Chrunyk, B., Cunningham, D., Flynn, D., Garofalo, R.S., et al., 2010. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. Journal of Biological Chemistry 285(11): 8340–8351.
[92] Bai, P., Canto, C., Oudart, H., Brunyanszki, A., Cen, Y., Thomas, C., et al., 2011. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metabolism 13(4):461–468.
[93] Ame, J.C., Schreiber, V., Niedergang, C., Apiou, F., Decker, P., Muller, S., et al., 1999. PARP-2, A novel mammalian DNA damage-dependent poly(ADP-ribose)polymerase. Journal of Biological Chemistry 274(25):17860–17868.
[94] Fang, E.F., Scheibye-Knudsen, M., Brace, L.E., Kassahun, H., Sengupta, T., Nilsen, H., et al., 2014. Defective mitophagy in XPA via PARP-1 hyper-activation and NAD +/SIRT1 reduction. Cell 157(4):882–896.
[95] Scheibye-Knudsen, M., Mitchell, S.J., Fang, E.F., Iyama, T., Ward, T., Wang, J., et al., 2014. A high-fat diet and NAD+ activate sirt1 to rescue premature aging in cockayne syndrome. Cell Metabolism 20(5):840–855.
[96] Chen, Y., Sprung, R., Tang, Y., Ball, H., Sangras, B., Kim, S.C., et al., 2007. Lysine propionylation and butyrylation are novel post-translational modifications in histones. Molecular & Cellular Proteomics 6(5):812–819.
[97] Tan, M., Luo, H., Lee, S., Jin, F., Yang, J.S., Montellier, E., et al., 2011. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 146(6):1016–1028.
[98] Xie, Z., Dai, J., Dai, L., Tan, M., Cheng, Z., Wu, Y., et al., 2012. Lysine succinylation and lysine malonylation in histones. Molecular & Cellular Proteomics 11(5):100–107.
[99] Tan, M., Peng, C., Anderson, K.A., Chhoy, P., Xie, Z., Dai, L., et al., 2014. Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. Cell Metabolism 19(4):605–617.
[100] Dai, L., Peng, C., Montellier, E., Lu, Z., Chen, Y., Ishii, H., et al., 2014. Lysine 2-hydroxyisobutyrylation is a widely distributed active histone mark. Nature Chemical Biology 10(5):365–370.
[101] Simithy, J., Sidoli, S., Yuan, Z.-F., Coradin, M., Bhanu, N.V., Marchione, D.M., et al., 2017. Characterization of histone acylations links chromatin modifications with metabolism. Nature Communications 8(1):1141.
[102] Ruiz-Andres, O., Sanchez-Niño, M.D., Cannata-Ortiz, P., Ruiz-Ortega, M., Egido, J., Ortiz, A., et al., 2016. Histone lysine crotonylation during acute kidney injury in mice. Disease and Molecular Medicine 9(6):633–645.
[103] Jenuwein, T., 2001. Translating the histone code. Science (80-.) 293(5532): 1074–1080.
[104] Kaczmarska, Z., Ortega, E., Goudarzi, A., Huang, H., Kim, S., Márquez, J.A., et al., 2016. Structure of p300 in complex with acyl-CoA variants. Nature Chemical Biology 13(1):21–29.
[105] Shimazu, T., Hirschey, M.D., Newman, J., He, W., Shirakawa, K., Le Moan, N., et al., 2013. Suppression of Oxidative Stress by β-Hydroxybutyrate, an endogenous Histone Deacetylase Inhibitor. Science (80-.) 339(6116):211–214.
[106] Xie, Z., Zhang, D., Chung, D., Tang, Z., Huang, H., Dai, L., et al., 2016. Metabolic regulation of gene expression by histone lysine β-hydroxybutyrylation. Molecular Cell 62(2):194–206.
[107] Goudarzi, A., Zhang, D., Huang, H., Roeder, R.G., Goudarzi, A., Zhang, D., et al., 2016. Dynamic competing histone H4 K5K8 acetylation and butyrylation are hallmarks of highly active gene article dynamic competing histone H4 K5K8 acetylation and butyrylation are hallmarks of highly active gene promoters. Molecular Cell 62(2):169–180.
[108] Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R., Van Immerseel, F., 2010. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutrition Research Reviews 23(2):366–384.
[109] Kebede, A.F., Nieborak, A., Shahidian, L.Z., Le Gras, S., Richter, F., Gomez, D.A., et al., 2017. Histone propionylation is a novel 1 mark of active chromatin. Nature Structural & Molecular Biology(October).
[110] Liu, B., Lin, Y., Darwanto, A., Song, X., Xu, G., Zhang, K., 2009. Identification and characterization of propionylation at histone H3 lysine 23 in mammalian cells. Journal of Biological Chemistry 284:32288–32295.
[111] Du, J., Zhou, Y., Su, X., Yu, J.J., Khan, S., Jiang, H., et al., 2011. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. Science 334(6057):806–809.
[112] Traut, T.W., 1994. Physiological concentrations of purines and pyrimidines. Molecular and Cellular Biochemistry 140(1):1–22.
[113] Knight, Z.A., Shokat, K.M., 2005. Features of selective kinase inhibitors. Chemistry & Biology 12(6):621–637.
[114] Wang, Y., Guo, Y.R., Liu, K., Yin, Z., Liu, R., Xia, Y., et al., 2017. KAT2A coupled with the α-KGDH complex acts as a histone H3 succinyltransferase. Nature.
[115] Frayling, T.M., Timpson, N.J., Weedon, M.N., Zeggini, E., Freathy, R.M., Lindgren, C.M., et al., 2013. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science (80-.) 889(2007):163–169.
[116] Jia, G., Fu, Y., Zhao, X., Dai, Q., Zheng, G., Yang, Y., et al., 2011. N6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nature Chemical Biology 7(12):885–887.
[117] Böhm, J., Schlaeger, E.-J., Knippers, R., 1980. Acetylation of nucleosomal histones in vitro. European Journal of Biochemistry 112(2):353–362.
[118] Lau, O.D., Courtney, A.D., Vassilev, A., Marzilli, L.A., Cotter, R.J., Nakatani, Y., et al., 2000. p300/CBP-associated factor histone acetyltransferase processing of a peptide substrate: kinetic analysis of the catalytic mechanism. Journal of Biological Chemistry 275(29):21953–21959.
[119] Wiktorowicz, J.E., Campos, K.L., Bonner, J., 1981. Substrate and product inhibition initial rate kinetics of histone acetyltransferase. Biochemistry 20(6): 1464–1467.
[120] Tuck, M.T., Farooqui, J.Z., Paik, W.K., 1985. Two histone H1-specific protein-lysine N-methyltransferases from Euglena gracilis. Purification and characterization. Journal of Biological Chemistry 260(11):7114–7121.
[121] Rathert, P., Zhang, X., Freund, C., Cheng, X., Jeltsch, A., 2008. Analysis of the substrate specificity of the Dim-5 histone lysine methyltransferase using peptide arrays. Chemistry & Biology 15(1):5–11.
[122] Melnyk, S., Pogribna, M., Pogribny, I.P., Yi, P., James, S.J., 2000. Measurement of plasma and intracellular S-adenosylmethionine and S- adenosylhomocysteine utilizing coulometric electrochemical detection: alterations with plasma homocysteine and pyridoxal 5'-phosphate concentrations. Clinical Chemistry 46(2):265–272.
[123] Kossykh, V.G., Schlagman, S.L., Hattman, S., 1995. Phage T4 DNA [N6-adenine]methyltransferase. Overexpression, purification, and characterization. Journal of Biological Chemistry 270(24):14389–14393.
[124] Del Gaudio, R., Di Giaimo, R., Potenza, N., Branno, M., Aniello, F., Geraci, G., 1999. Characterization of a new variant DNA (cytosine-5)-methyltransferase unable to methylate double stranded DNA isolated from the marine annelid worm Chaetopterus variopedatus. FEBS Letters 460(2):380–384.
[125] Simon, D., Grunert, F., Acken, U. v., Döring, H.P., Kröger, H., 1978. DNA-methylase from regenerating rt liver: purification and characterisation. Nucleic Acids Research 5(June):2153–2168.
[126] Cohen, H.M., Griffiths, A.D., Tawfik, D.S., Loakes, D., 2005. Determinants of cofactor binding to DNA methyltransferases: insights from a systematic

series of structural variants of S-adenosylhomocysteine. Organic and Biomolecular Chemistry 3:152–161.

[127] Guan, X., Lin, P., Knoll, E., Chakrabarti, R., 2014. Mechanism of inhibition of the human sirtuin enzyme SIRT3 by nicotinamide: computational and experimental studies. PLoS One 9(9).

[128] Borra, M.T., Langer, M.R., Slama, J.T., Denu, J.M., 2004. Substrate specificity and kinetic mechanism of the Sir2 family of NAD +-dependent histone/protein deacetylases. Biochemistry 43(30):9877–9887.

[129] Yamada, K., Hara, N., Shibata, T., Osago, H., Tsuchiya, M., 2006. The simultaneous measurement of nicotinamide adenine dinucleotide and related compounds by liquid chromatography/electrospray ionization tandem mass spectrometry. Analytical Biochemistry 352(2):282–285.

[130] Belenky, P., Racette, F.G., Bogan, K.L., McClure, J.M., Smith, J.S., Brenner, C., 2007. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via nrk and urh1/pnp1/Meu1 pathways to NAD+. Cell 129(3):473–484.

[131] Yang, H., Yang, T., Baur, J.A., Perez, E., Matsui, T., Carmona, J.J., et al., 2007. Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. Cell 130(6):1095–1107.

[132] Cascella, B., Mirica, L.M., 2012. Kinetic analysis of iron-dependent histone demethylases: α-Ketoglutarate substrate inhibition and potential relevance to the regulation of histone demethylation in cancer cells. Biochemistry 51(44):8699–8701.

[133] Chowdhury, R., Yeoh, K.K., Tian, Y.M., Hillringhaus, L., Bagg, E.A., Rose, N.R., et al., 2011. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Reports 12(5):463–469.

[134] Rose, N.R., Ng, S.S., Mecinovic, J., Lienard, B.M., Bello, S.H., Sun, Z., et al., 2008. Inhibitor scaffolds for 2- oxoglutarate-dependent histone lysine demethylases. Journal of Medicinal Chemistry 51(ll):7053–7056.

[135] Chin, R.M., Fu, X., Pai, M.Y., Vergnes, L., Hwang, H., Deng, G., et al., 2014. The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature 510(7505):397–401.

[136] Shen, J., Khan, N., Lewis, L.D., Armand, R., Grinberg, O., Demidenko, E., et al., 2003. Oxygen consumption rates and oxygen concentration in molt-4 cells and their mtDNA depleted (ρ^0) mutants. Biophysical Journal 84(2 Pt 1):1291–1298.

[137] Laukka, T., Mariani, C.J., Ihantola, T., Cao, J.Z., Hokkanen, J., Kaelin, W.G., et al., 2016. Fumarate and succinate regulate expression of hypoxia-inducible genes via TET enzymes. Journal of Biological Chemistry 291(8):4256–4265.

[138] Koivunen, P., Lee, S., Duncan, C.G., Lopez, G., Lu, G., Ramkissoon, S., et al., 2012. Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 483(7390):484–488.

