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. EPGENETIC 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
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 stage, 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 histone methylation 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 catalyzed 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 (m6A) is the most prevalent in eukaryotic mRNA [32]. Although studies have reported different effects of m6A on RNA functions and correlations between alterations in m6A 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

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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 homo-cysteine (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\text{vy}), depending on the CpG methylation level of the IAP retrotransposon; high methylation correlates with high expression of A\text{vy} expressed in liver, encodes the \text{MAT1A} homologous, but different, \text{MAT} catalytic subunits. There are 3 \text{MAT} isozymes in mammals (Figure 2E). Two genes encode the catalytically inactive, regulatory subunit of \text{MAT} isozymes, which are either a tetramer (\text{MAT I}) or dimer (\text{MAT III}) of this subunit found in two native \text{MAT} isozymes in mammals (Figure 2E). Two genes encode homologous, but different, \text{MAT} catalytic subunits. \text{MAT1A}, mostly expressed in liver, encodes the x1 subunit found in two native \text{MAT} isozymes, which are either a tetramer (\text{MAT I}) or dimer (\text{MAT II}) of this subunit. \text{MAT2B} is expressed in many tissues including brain, kidney, testis, lymphocytes, foetal liver and, to a lesser extent, adult liver. The catalytic subunit it encodes (x2) is found in a native \text{MAT} isozyme (\text{MAT II}) associated with the catalytically inactive, regulatory subunit x1 encoded by \text{MAT2B}.

Impaired enzymatic activity of \text{MAT} isozymes has a profound effect on histone methylation in many organisms. Depletion of \text{MAT2B} in immortalised mouse embryonic fibroblasts (iMEF) significantly reduces trimethylation 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.
types of cancer, catalyse iterative conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by Ten-eleven translocation (TET) enzymes, previously associated with translocations in some cancers. The discovery that Ten-eleven translocation enzymes modify DNA at the promoter of target genes was a major breakthrough in understanding the role of epigenetic modifications in cancer.

For many years there was a notion that the loss of methyl marks on C-5 of cytosine occurred through passive dilution during DNA replication and repair processes. The discovery that Ten-eleven translocation (TET) enzymes, which are a class of 2-oxoglutarate-dependent dioxygenases, can catalyse the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) through a mechanism involving the enzyme catalysing the conversion of isocitrate to α-ketoglutarate (α-KG), has revolutionized our understanding of DNA methylation.

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 trimethylated Lys.

4.2. Fe²⁺ and α-ketoglutarate dependent dioxygenases can also demethylate histones

The largest class of histone demethylases are Jumonji C (JmjC) domain-containing enzymes, which belong to the bigger family of Fe²⁺ and α-KG-dependent dioxygenases (2-OGDO) and are grouped into several subfamilies. The catalytic mechanism is based on 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 of cytosine occurred through passive dilution during DNA replication and repair processes. The discovery that Ten-eleven translocation (TET) enzymes, previously associated with translocations in some types of cancer, catalyse the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) has revolutionized our understanding of DNA methylation.

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 KDM4L [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 post-translationally acetylated at their ε-amino group by N-terminal acetyltransferases. The second major type of acetylation occurs on the ε-amino group of Lys residues. The first-comprehensive evidence of Lys acetylation as a post-translation modification 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 (HDACs). Up to now, acetyl groups have been found on more than 60 histone Lys residues. In the epigenetics field, therefore, KATs and HDACs 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 pl of Lys (pLys = 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 E1x1 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 changing phase; c. reprinted from [58] with permission from the authors and ELSEVIER.

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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 are caused by increased Tdh expression in mESCs but whether this is linked to the metabolic state of the cell, is a crucial factor regulating histone acetylation levels. In mammalian cells, increased acetyl-CoA availability, which re

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$^+$ levels. 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 a metabolic readout in 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 nuclease-cytosolic (SIRT1, SIRT2, SIRT6, and SIRT7) and three mitochondrial (SIRT3, SIRT4, SIRT5) [78–80]. SIRT activity is inhibited by several exogenous small molecules (sirtomicin, 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.
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 status 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

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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 (NMM) as an intermediary product; CD38 — cyclic ADP-ribose hydrolase; IDO — indoleamine 2,3-dioxygenase; NAD — nicotinic acid adenine dinucleotide; NADS — nicotinamide adenine dinucleotide synthetase; NAM — nicotinic acid mononucleotide; NAPT — nicotinic acid phosphoribosyltransferase; PARPs — poly(ADP-ribose) polymerase; QAPRT — quinolinate phosphoribosyltransferase; SIRTs — sirtuins.
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 catabolic pathways, the regulation of which depends on the energetic state of the cell) resulting in decreased acetyl-CoA concentrations and transferring this information to chromatin via distinct histone acylations.

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 transcription, sustaining transcription of genes escaping MSCI in spermatogenesis | [107] |
| 2-Hydroxyisobutyrylation | Polar                   | Both N-terminal tails and globular domains | BCAA catabolism | Gene activation | [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] |
| 2-Hydroxybutyrylation | Polar                       | Both N-terminal tails and globular domains | SCFA oxidation, ketogenesis | Starvation induced gene activation | [106] |

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 Km for ATP is significantly 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 Km for ATP is significantly 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.
Histone acetyltransferases, acyl-CoA CoA, acyl-CoAs 0.2–46 2–20/? Cytoplasm, mitochondria, nucleus [71,101,117] [118,119]

Histone methyltransferases DNA methyltransferases SAM SAM SAH, MTA 1.2–34.5 3.3–59/? Cytoplasm, nucleus [120–122] [122,123]

Histone acetyltransferases’ Sirtuins acyl-CoA CoA, acyl-CoAs 0.1–21 3.3–59/? Cytoplasm, nucleus [124–126] [101]

Histone demethylases, 2-OGDO α-KG R-2HG, succinate, fumarate 9–37 110–260/? Cytoplasm, mitochondria [53,132–135]

Lsd1/2 DNA demethylation FAH FAH 35–75 110–260/? Cytoplasm, mitochondria [135–138]

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++ and α-KG dependent dioxygenases; * — histone acetyltransferases with reported acyltransferase activity; p300, MOF, PCAF, GCN5, TIP60.

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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. 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 m5A, 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 metabolopigenic 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 metabolopigenetics will lead us to a better understanding of these reciprocal links.

CONFLICT OF INTEREST

None.

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