Histone acylations and chromatin dynamics: concepts, challenges, and links to metabolism

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Abstract

In eukaryotic cells, DNA is tightly packed with the help of histone proteins into chromatin. Chromatin architecture can be modified by various post-translational modifications of histone proteins. For almost 60 years now, studies on histone lysine acetylation have unraveled the contribution of this acylation to an open chromatin state with increased DNA accessibility, permissive for gene expression. Additional complexity emerged from the discovery of other types of histone lysine acylations. The acyl group donors are products of cellular metabolism, and distinct histone acylations can link the metabolic state of a cell with chromatin architecture and contribute to cellular adaptation through changes in gene expression. Currently, various technical challenges limit our full understanding of the actual impact of most histone acylations on chromatin dynamics and of their biological relevance. In this review, we summarize the state of the art and provide an overview of approaches to overcome these challenges. We further discuss the concept of subnuclear metabolic niches that could regulate local CoA availability and thus couple cellular metabolisms with the epigenome.

Keywords acylation; chromatin; histones; metabolism; microdomains

Introduction: Histone lysine acylations and metabolism

Genomic information is stored in the nucleus of eukaryotic cells in a structure referred to as chromatin which consists of DNA and proteins (Ollins & Ollins, 2003). The smallest building blocks of chromatin are nucleosomes, which are composed of 147 base pairs of DNA wrapped around a histone octamer. Such a histone octamer is formed by one histone H3-H4 tetramer and two histone H2A-H2B dimers. The flexible N-termini as well as the globular core domain of these four core histones can be heavily covalently modified (Kornberg, 1974; Lugier et al., 1997). The best studied histone post-translational modifications (PTM) are currently acetylation (Kuo & Allis, 1998), methylation (Kouzarides, 2002), and phosphorylation (Oki et al., 2007). It has been suggested that these PTMs could form a so-called histone code (Strahl & Allis, 2000). If it is indeed a “code” is still controversial, but it becomes more and more convincing that the complex diversity of PTMs enables fine tuning of chromatin structure and function (Rando, 2012). Since chromatin regulates DNA accessibility, changes in chromatin structure influence DNA-dependent processes such as transcription (Tropberger & Schneider, 2013; Tessarz & Kouzarides, 2014). How chromatin architecture is regulated by histone PTMs can currently be best explained for histone lysine acetylation (Kac).

Positively charged lysine residues, for instance in the histone H4 tail, can interact with negatively charged DNA or neighboring nucleosomes. Acetylation of the ε-amino group of lysines occurs on many lysines in histone tails and globular domains and is catalyzed by histone acetyltransferases (HAT). Acetylation neutralizes the positive charge of lysines and thus can weaken the interactions between histones and DNA, contributing to a more open chromatin state (Fig 1A). Higher DNA accessibility enables, for example, transcription factor binding and can promote transcriptional activity (Bannister & Kouzarides, 2011). In addition, to these direct effects, Kac can also be directly bound by specific interactors (so-called “reader” proteins), such as bromodomain-containing (BRD) transcription factors that promote gene expression (Wang et al., 2007). Genome-wide distribution studies show a positive correlation between enrichment of histone acetylations at transcriptional start sites (TSS) and gene expression (Hebbes et al., 1988; Mikkelsen et al., 2007). Transcriptional regulation via Kac seems to be critical for cellular function, as aberrant acetylation is linked to cancer development, neurological disorders, and also metabolic diseases (Timmermann et al., 2001; Zhong & Kowluru, 2010; Sheikh, 2014).

Kac on the histone tails has a dynamic turnover and is dependent on the availability of the acetyl donor, acetyl-CoA, which is a key metabolic intermediate. Acetyl-CoA can diffuse from the cytoplasm to the nucleus or can be locally produced in the nucleus (preprint: Kafkia et al., 2020). Cellular changes in the acetyl-CoA concentration can be reflected in the levels of histone acetylation (Sivanand et al., 2021).
2018). Intriguingly, metabolic enzymes that produce acetyl-CoA such as the pyruvate dehydrogenase complex (PDC), ATP citrate lyase (ACLY), acyl-coenzyme A synthetase 2 (ACSS2), or the carnitine acetyltransferase (CRAT) can localize in the nucleus, which can lead to increased nuclear Kac levels (Boukouris et al., 2016). Additionally, ACLY as well as the ACSS2 homolog in Saccharomyces cerevisiae were shown to be involved in the regulation of histone acetylation levels (Takahashi et al., 2006; Wellen et al., 2009). More recently, Mews et al. (2017) linked reduced ACSS2 protein amounts with decreased nuclear acetyl-CoA concentrations and decreased histone acetylation levels as well as reduced gene expression (Mews et al., 2017). Such findings provide insights into how cellular metabolism, via cofactors required by chromatin modifiers, can “talk” to the epigenome and gave rise to the new field of metaboloepigenomics. How exactly this local acetyl-CoA production is established and how local acetyl-CoA pools are maintained is still one of the major questions in the field (Katada et al., 2012).

Findings that other acyl-CoAs besides acetyl-CoA can occur in the nucleus and function as acyl donors for various histone-modifying enzymes, added additional complexity to the panel of histone modifications (Pietrocola et al., 2015; preprint: Trefely et al., 2020a). In line with this, metabolic enzymes involved in the generation of these additional acyl-CoAs have been recently detected in the nucleus. For example, the nuclear α-ketoglutarate dehydrogenase (KGDH) complex can increase the concentration of nuclear succinyll-CoA (Wang et al., 2017). Due to the development of increasingly sensitive mass spectrometry (MS) techniques, also novel types of histone lysine acylations and new acylation sites have been identified (Fig 1B). These lysine acylations include 2-hydroxyisobutyrylation (Khib) (Xie et al., 2016), benzyoylation (Kbz) (Huang et al., 2018c), butyrylation (Kbu) (Chen et al., 2007), isobutyrylation (Kibu) (Zhu et al., 2021), crotonylation (Kcr) (Tan et al., 2011), glutarylation (Kglu) (Bao et al., 2019), lactylation (Kla) (Zhang et al., 2019a), malonylation (Kma) (Xie et al., 2012), propionylation (Kpr) (Chen et al., 2007) and succinylation (Ksuc) (Xie et al., 2012). Since the acyl-CoAs required for these modifications are derived from different metabolic pathways, specific histone acylations could act as sensors of the metabolic state of a cell and fine tune chromatin architecture and thus gene expression according to cellular needs (Simithy et al., 2017; Trefely et al., 2020b).

The versatility of the different histone lysine acylations is determined by their distinct chemical properties. Only acidic modifications like Ksuc, Kmal, and Kglu change the positive lysine charge to a negative one. The branched 4-carbon and polar modifications Khib and Kbhb contain hydroxyl groups and enable modified lysines to form additional hydrogen bonds. Hydrophobic acylations include Kcr, Kbu, Kbz, and Kpr. Kpr (linear) is a 3-carbon, Kbu (linear), and Kcr (planar) are 4-carbon modifications. Kbz is currently the only described histone acylation with an aromatic acyl group. Increasing the acyl chain length further increases the hydrophobicity and also the steric hindrance potential of modified lysines (Sabari et al., 2017; Dai et al., 2020). This short summary highlights the diversity of acylations that can occur on histone lysines.

Multiple studies have suggested functional differences for some of these acylations and have been excellently reviewed elsewhere (Sabari et al., 2017; Li et al., 2018; Barnes et al., 2019; Boon et al., 2020; Dai et al., 2020; Haws et al., 2020; Trefely et al., 2020b). However, important questions still need to be addressed to fully understand the impact of metabolism on cellular function via chromatin-based mechanisms. For this, additional mechanistic insights into the effect of acylations on chromatin structure and function will be necessary. In the last years, new highly sensitive and well-controllable assays have been developed that can help to mechanistically understand the role of additional lysine acylations in gene expression, and how different acylations compare to each other (Cuivia & Fierz, 2017). In this review, we aim to provide an overview of different strategies that can be used to deepen our mechanistic insight into lysine acylations and to discuss the findings gained with these approaches. We will further focus on the principle
Site-specific mutations of histones have been used to mimic modifications or the unmodified state. One advantage of such mimics is that they can be used in vitro and in vivo. For example, histone point mutants have been used to study the function of acylations in Saccharomyces cerevisiae. The advantage of lower eukaryotes is that it is relatively simple to replace all endogenous histones with the mutated ones. For example, H2BK37E (as a K37succ mimic), H2AK119E (as a K119mal mimic), and H4K91E (as a H4K91glu mimic) mutant yeast strains were used to investigate chromatin structure changes in vivo and complemented existing in vitro data nicely (Ishiguro et al., 2018; Jing et al., 2018; Bao et al., 2019). Nevertheless, no mimic can entirely resemble the actual modification and close structural similarity of some short-chain lysine acylations such as Kac, Kpr, or Kbu makes it difficult to mimic acylations specifically (Tropberger et al., 2013; Zorro Shahidian et al., 2021). Furthermore, these mimics are constant alterations of histones, which is in contrast to the rather dynamic nature of endogenous acylations (Katan-Khaykovich, 2002). "Genetically encoding" acetyl-lysine can enable the generation of proteins acylated at defined sites (Fig 2A). For example, the amber (stop) codon suppression by an orthogonal acetyl-lysyl-tRNA synthetase (ACKRS) tRNA pair allows for the incorporation of noncanonical amino acids (e.g., acetylated lysine) into proteins (Neumann et al., 2008). A high yield of site-specifically acylated histones can be successfully obtained, e.g., by their expression in E. coli. Another common approach to obtain site-specific acylated nucleosomes is via a native chemical ligation (NCL) or expressed protein ligation (EPL), in which peptides containing C-terminal thioesters are fused to an N-terminal cysteine from another peptide (Dawson et al., 1994; He et al., 2003). For example, histone tails with acylated lysines can be generated by peptide synthesis and truncated histones can be recombinantly expressed and used for the chemo-selective reaction (Fig 2A) (Shogren-Knaak, 2006). This semi-synthetic method allows for the incorporation of multiple modifications on the same histone tail, as well as of different types of acylations. Alternatively, complete chemical synthesis of histones allows multiple distinct acylations to be added, but remains elaborate and inefficient in comparison with the amber system or NCL/EPL approaches (Zorro Shahidian et al., 2021). Eissasser et al. (2016) demonstrated that site-specific histone acetylation can be genetically encoded in mammalian cells by stable integration of the components of the amber system and deposited into chromatin. However, cellular deacetylases can act quickly on these sites resulting in deacetylation, a problem that most likely also applies to other types of acylations. Recently, Fujiwara et al. (2021) demonstrated that site-specific acylations can also be introduced in vivo via a protease-resistant nucleosome-binding catalyst and a cell-permeable acyl donor. Subsequent development of this exiting method might enable us to study the effects of histone acylations on nucleosome assembly in vivo.

Impact of histone acylations on chromatin structure

Chromatin accessibility and compaction can be fine-tuned by an interplay of various histone PTMs including different types of acylations (Trefely et al., 2020b). However, our mechanistic understanding of the direct or indirect effects of acylations on chromatin is still limited. In order to gain more insight, in vitro assays have been used, many of which require so-called "designer" chromatin. Designer chromatin with specific histone acylations facilitates highly controllable in vitro studies on the direct effects of these acylations. To generate acylated nucleosomes or chromatin, several different approaches have been applied (Müller & Muir, 2015). More details on the generation of such chromatin are provided in Box 1 and Fig 2A. Although most of the previous studies focused on histone acetylation, we still discuss some of them here to encourage similar mechanistic analyses for other types of acylations.

Chromatin dynamics can be shaped by nucleosome stability, which can be directly affected by acetylations on the core domain of histones (Tropberger & Schneider, 2013). To reveal the influence of acylated lysines in the histone core region on nucleosome stability, Bao et al. (2019) investigated histone H4 glutaryllylation at lysine 91. During octamer purification via size-exclusion chromatography, they observed different elution profiles for H4K91glu octamers and unmodified octamers, hinting toward inefficient octamer assembly upon H4K91glu. This destabilization of nucleosomes provided mechanistic insight into the role of H4K91glu at highly expressed genes (Bao et al., 2019). Furthermore, they showed that H4K91glu facilitates H2A/H2B dimer dissociation from nucleosomes using a fluorescence resonance energy transfer (FRET) assay. Such FRET assays are well suited to study nucleosome stability but also to analyze folding of nucleosomal arrays (Fig 2B). Via a FRET approach, a destabilization effect on nucleosomes upon succinylation of H3K122, again located within the globular domain, was revealed (Zorro Shahidian et al., 2021). Likewise, H4K77succ was shown to decrease nucleosome stability while nucleosomal DNA accessibility increased (Jing et al., 2020). In addition to site-specific acylations, global Kac, Kpr, Kbu, and Kmal of histones can weaken nucleosome stability, histone-DNA interactions and promote nucleosome sliding. Of these modifications, Ksuc has the lowest acetyl-CoA chain and imparts a negative charge, which might explain why it was able to destabilize nucleosomes to the greatest extent in an optical tweezer approach using 12-mer nucleosomal arrays (preprint: Smestad et al., 2020). In general, single-molecule force spectroscopy techniques, such as optical or magnetic tweezers, can be used to study the stability of clamped mononucleosomes and nucleosomal arrays by applying an accurate force and measuring motions (Fig 2C) (Neuman & Nagy, 2008).

For studies on the impact of acylations on chromatin structure, the histone H4 tail can serve as a convenient starting point. The H4 tail plays an important role in this regard, since it can directly interact with the acidic patch (created between H2A/H2B) of the neighboring nucleosome (Luger et al., 1997). Kac in the H4 tail is one of the few histone modifications that has been shown to disrupt inter-nucleosomal interactions and has a direct impact on chromatin structure (Shogren-Knaak, 2006; Allahverdi et al., 2011). This structural effect is not solely caused by charge neutralization of H4K16 and reduced interaction with the neighboring nucleosome. Collepardo-Guevara et al. (2015) observed that upon K16 acetylation, histone H4 tail flexibility decreases. This loss of flexibility could additionally limit internucleosomal interactions (Collepardo-Guevara et al., 2015) and thus provide another perspective on how H4K16ac opens up chromatin. This insight was based on nuclear magnetic resonance (NMR) spectroscopy, which enables analysis of nucleosome structure and tail dynamics in combination with computational simulation studies (Musselman & Kutateladze, 2021). Similar approaches could be used to systematically compare the effect of non-acetyl acylations on histone tail dynamics for all core of metabolic microdomains in the nucleus as well as the current challenges to study metabolopigenomics in vivo.
A loss of nucleosomal array compaction upon H4K16ac was also observed in a more direct manner by using designer chromatin and analytical ultracentrifugation (AUC) sedimentation velocity experiments (Shogren-Knaak, 2006). Similar AUC assays could be useful for further comparison of the impact of different acylations on assembled chromatin (Shogren-Knaak, 2006; Funke et al., 2016). Interestingly, these effects seem to be specific to H4K16 modifications, since the acetylation of the nearby lysine H4K20 does not interfere with chromatin compaction (Wilkins et al., 2015). This could be explained by changes in the internucleosomal interactions caused by H4K16ac as demonstrated by Funke et al., (2016). For this, the authors developed an elegant method using a DNA origami-based force spectrometer (Fig 2E). This positioning device is based on a nanoscale folding of DNA with a flexible hinge region (stapler like) that allows for the targeted incorporation of mononucleosomes and the measurement of internucleosomal interactions strength (Funke et al., 2016). To assess the influence of histone acylation on chromatin structure in vivo is technically very challenging. Sidoli et al., (2019) provided a first step in this direction via a sophisticated approach to analyze the influence of histone PTMs on chromatin accessibility by MS and metabolic labeling. This method could also be useful to analyze acylation-dependent chromatin compaction in cellulo (Sidoli et al., 2019).

To summarize, many of these in vitro assays will be extremely useful for studies on the specific effects of histone acylation, beyond acetylation, in the future. A combination of different assays coupled with chromatin simulation approaches could help to precisely predict chromatin behavior upon specific histone acylation events (Moller & de Pablo, 2020). The ultimate goal is to study chromatin structure dynamics in vivo, as nucleosomal arrays do not resemble the complexity of chromatin in the nucleus. Recent advances in 3D super-resolution microscopy hold the promise to examine the

**Figure 1. Overview of lysine acylations on core histones and their role in chromatin compaction.**

(A) Increasing histone acylation levels can contribute to opening up chromatin. (B) Identified lysine acylation sites in the four core histones (Sabari et al., 2017; Barnes et al., 2019; Zhang et al., 2019). Lysines within the N-terminal histone tail are in bold. Selected acylations and their chemical nature are depicted (hydrophobic: blue, polar: gray, acidic: red). Abbreviations: ac—acetylation, pr—propionylation, bu—butyrylation, cr—crotonylation, bz—benzoylation, hib—2-hydroxyisobutyrylation, bhb—β-hydroxybutyrylation, la—lactylation, mal—malonylation, succ—succinylation, glu—glutarylation.
influence of histone acylations on chromatin compaction in vivo (Otterstrom et al., 2019).

**Acylation-specific readers, writers, and erasers**

With the identification of novel types of histone acylations, the question arose whether canonical HATs are placing these diverse acylations or if additional, yet unidentified, acyltransferases exist. The same considerations hold true for the erasers, the histone deacetylases (HDAC). Also, many questions concerning acyl-specificity of “reader” proteins remain open (Allis & Jenuwein, 2016). So far, very few systematic screens have been performed to identify new acylation-specific writers, readers, or erasers, in an unbiased way (Fig 2F).

Most in vitro and in vivo studies have focused on the role of known HATs in mediating other types of acylations. (Zhao et al., 2018). For example, in vitro HAT assays revealed that several classical HATs such as KAT2A (GCN5), KAT3B (p300), Tip60, or MOF can use various acyl-CoAs (pr-/ bu-/ cr-/ mal-/ bhb-/ succ- and glu-CoA) as their cofactors, but for many of these enzymes the acyltransferase activity seems to decrease for bulkier acyl-CoAs. KAT3B, for example, seems to function as an acyltransferase “allrounder”, being able to catalyze H3K18hib, H4K8hib (Huang et al., 2018a), H4K5bu, H4K8bu (Goudarzi et al., 2016), H3K14pr, H3K14bu (Kebbede et al., 2017), H3/H4K18b (Kaczmarska et al., 2017), H3/H4K12 (Zhu et al., 2021), and H3K12s (Zorro Shahidian et al., 2021). Kaczmarska and colleagues showed that the KAT3B (p300) activity decreases with increasing acyl chain length since longer
acyl-CoAs interfere with histone lysine binding (Kaczmarska et al., 2017). Additionally, screenings of classical HATs for their activity toward different acylations also revealed that some HATs have a higher affinity toward other acetyl-CoAs than acetyl-CoA. For instance, KAT2A (GCN5) has a higher binding affinity for succinyl-CoA than for acetyl-CoA (Wang et al., 2017). It should also be considered that in vivo additional cellular factors (absent in in vitro assays) could enhance the ability of, e.g., KAT3B to use specific acyl-CoAs, probably by inducing some structural rearrangements. Furthermore, the high reactivity of acyl-CoAs can also cause acylations on histones independently of enzymatic mechanisms (Trub & Hirschey, 2018) resulting in non-enzymatic covalent modifications (NECMs). These NECMs are an under-studied class of post-translational modifications that add further complexity to the control of histone acylations and need to be carefully considered for instance for in vivo studies (see next section) (Simithy et al., 2017).

Similar to these findings with HATs, studies with HDACs have revealed that classical HDACs can have activity toward additional histone acylations. For example, class I HDACs can also act as histone deacytoylases in vitro and in vivo (Fellows et al., 2018). In addition, HDAC2 and HDAC3 were identified as the major enzymes to erase Kib (Huang et al., 2018a). Activity profiling on siRNAs (SIRT1-7) on multiple H3K9 acylations revealed that most siRNAs can remove different acylations (K9ac, K9bu, K9cr, K9bhb on H3). Interestingly, only SIRT2 and SIRT5 acted on H3K9succ. Furthermore, this profiling revealed SIRT1-3 and SIRT5 as novel de-β-hydroxybutyrylases (Zhang et al., 2019b).

Regarding reader domain specificity, most human bromodomains such as BRD2 or BRD4 seem to have higher affinities for Kcr than Kcr or Kbu. In contrast, bromodomains with larger binding pockets seem to be more selective for Kpr than other acylations (Flynn et al., 2015). As for other enzymatic modifications, the YEATS domain of CDYL seems to impact its enzymatic function (Caron et al., 2003). Upon crotonyl-CoA binding, CDYL was shown to act as a crotonyl-CoA hydrolase and to be involved in the reduction of histone Kcr levels (by converting crotonyl-CoA into β-hydroxybutyryl-CoA) (Liu et al., 2017) whereas the interaction of HDAC1/2 with CDYL prevents CoA binding. But in both cases, CDYL seems to play a part in transcriptional repression: in one scenario as a metabolic enzyme and in the other one as an epigenetic regulator (Caron et al., 2003; Liu et al., 2017). Findings like this highlight the tight links between metabolic processes and epigenetic mechanisms. We will discuss this crosstalk between histone acylation and cellular metabolism in more detail below.

### Influence of histone acylations on transcription: mechanisms and correlations

The links between histone acylations and chromatin structure described above suggest that at least some acylations might be involved in transcriptional regulation. To investigate the effect of histone acylation on transcription, highly controllable in vitro transcription (IVT) assays on designer chromatin have been developed (Fig 2G) (Sawadogo & Roeder, 1985) using either nuclear extracts as the source for polymerases and cofactors or only purified, recombinant components. Applying such an IVT system, Kebede et al., (2017) showed that Kpr can enhance transcription (Kebede et al., 2017). Furthermore, Goudarzi and colleagues (2016) observed that Kbu can activate transcription to at least a similar extent as Kac (Goudarzi et al., 2016). Kcr has been suggested to stimulate transcription to a greater extent than acetylation (Sabari et al., 2015), while Kib (Huang et al., 2018b) and Kla (Zhang et al., 2019a) enhance transcription to a lesser extent than Kac. Designer chromatin has been used to demonstrate how two acylations, Kac (Troppberger et al., 2013) and Kbu (Zorroz Shahidian et al., 2021), on the same residue on the lateral surface of the histone octamer (H3K122) lead to a similar enhancement of transcription. To study the dynamics of transcription, a new innovative variation of the IVT assay, which enables real-time detection of the transcripts by fluorescence correlation spectroscopy, has recently been developed.

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Via this approach, it was possible to demonstrate that tetra-acetylated H4 (acetylated at K5, K8, K12, and K16) stimulates transcription in vitro and to provide a model for the contribution of these acetylations to different steps of the transcription process (Wakamori et al., 2020). Overall, in vitro transcription assays are a powerful tool, which can dissect the precise impact of specific histone acylations on transcription.

In contrast to these more mechanistic in vitro approaches, intriguing insights into the correlations between acylations and transcription have emerged from genome-wide histone acylation enrichment studies (chromatin immunoprecipitation followed by high-throughput sequencing, ChIP-seq) in combination with gene expression profiling. However, these ChIP-seq approaches depend heavily on high-quality, ChIP-grade, site-specific antibodies, which are often limiting. The potentially high cross-reactivity of anti-acetyl antibodies makes it essential to extensively verify their specificity (Simithy et al., 2017) (see Box 2). In many studies, “pan” antibodies have been used that recognize a specific acylation type but are not acylation site or protein specific. Nevertheless, such antibodies can help to circumvent the lack of antibodies that can site-specifically recognize acylations on histone lysines. For instance, Tan et al., (2011) performed ChIP-seq with a pan anti-Kcr antibody and revealed Kcr enrichment at promoter and enhancer regions, which strongly overlapped with Kac signals in a resting somatic human cell line. In post-meiotic male germ cells (spermatids), however, regions on sex chromosomes were enriched in histone Kcr and low in Kac. This occurred at X/Y-linked genes that are active in post-meiotic male germ cells. The authors suggest that a specific ratio of Kcr and Kac could contribute to an escape from inactivation and to maintain the genes active, despite the general transcriptional repression of sex chromosomes in these cells (Tan et al., 2011). In addition, ChIP with a pan-Ksucc antibody revealed that Ksucc peaks strongly correlate with peaks for histone modifications that mark actively transcribed chromatin, such as H3K4me3. Upon loss of the enzyme succinate dehydrogenase (SDH) in irradiated mouse embryonic fibroblasts, Ksucc levels both increased and decreased at distinct promoters. These changes in Ksucc correlated with gene expression changes, suggesting, a gene-specific transcriptional control by Ksucc (Smes-tad et al., 2018). These studies show that using pan-acyl antibodies can provide insights into the relationship between acylation and transcription. However, the insight is limited as the antibodies might recognize all proteins carrying a specific acylation, both histone and non-histone, rendering it difficult to identify the specific contribution of a given histone acylation. Furthermore, not all acylation sites are equally well recognized by these antibodies due to epitope preferences and thus preference toward specific proteins.

In contrast to pan-acyl antibodies, site-specific antibodies allow to focus on one specific acylation site within a specific protein. With this higher precision, it is possible to study the effect of metabolic changes on specific histone acylations at defined gene regions. For instance, in order to understand the microbiome-host interaction better, Fellows et al., (2018) performed ChIP-seq in murine colon epithelial crypts with an anti-H3K18cr antibody, and linked H3K18cr enrichment at TSS to transcriptional activation (Fellows et al., 2018). Further, microbiota depletion via antibiotics in mice caused short-chain fatty acid loss, which decreased H3K18cr levels in the colon. These findings suggest that Kcr levels in the colon could reflect short-chain fatty acid generation by the microbiota and provide an example of how changes in metabolism can influence the epigenome (Fellows et al., 2018). However, whether this crosstalk is direct or rather indirect requires further investigation. Another way to address the crosstalk between histone acylation and metabolism is via metabolic challenges such as fasting/ starvation experiments. For instance, H3K9bhb levels increased in livers of fasted mice and were linked with activated transcription of genes involved in the starvation response (Xie et al., 2016). This finding might indicate a fine tuning of transcriptional regulation via Kbbh during metabolic challenges. In addition, H3K14pr and H3K14bu were implicated in the expression of genes involved in lipid metabolism pathways in livers of fasted mice (Kebede et al., 2017). Interestingly, metabolic enzymes that are involved in the synthesis of CoAs have also been found to be present at the same genomic regions as the corresponding histone acylations. For example, H3K79succ enrichment profiles correlate with the presence of α-KGDH, suggesting a functional connection between α-KGDH, K3K79succ, and transcriptional activity. This is supported by the finding that α-KGDH interacts with the histone acetyltransferase KAT2A that can succinylate histones, and that depletion of KAT2A results in a strong reduction of H3K79succ levels (Wang et al., 2017). In contrast to these examples of positive correlations between lysine acylations and gene expression, a noteworthy finding was recently made by
Gowans et al, (2019). They showed that H3K9cr was linked to a reduced expression of growth genes during low-nutrient periods in Saccharomyces cerevisiae. The authors speculate that this unexpected H3K9cr-mediated repression might derive from disruption or inhibition of transcriptional initiation via Taf14 recruitment (Gowans et al, 2019). An explanation for these results could be that the transcriptional regulation via histone acylations is defined by the relative abundance of non-acetyl acylations (in this case crotonylation) compared with acetylation. For instance, changes of the acylation/acetylation ratios in a given promoter region could determine the transcriptional activity through dynamic recruitment or repulsion of specific interactors. The observed transcriptional repression by Gowans and colleagues could therefore be caused by an acylation/acetylation ratio shifted toward Kcr and thus recruitment of Taf14 (Gowans et al, 2019). A similar model to explain the functional diversity of histone acylations has been proposed by Goudarzi et al, (2016). Their study revealed that although the Brdt protein, a testis-specific BET bromodomain factor, cannot bind H4K5bu in vitro, in vivo it was found at genomic sites where both H4K5ac (bound by Brdt) and H4K5bu co-exist (Goudarzi et al, 2016). The dynamic exchange of acylation marks and alterations of their ratios locally could allow for dynamic interactions of Brdt with chromatin. The importance of the acylation/acyetlation ratio is further supported by the fact that most of the described ChIP-seq datasets are pointing toward the genomic co-occurrence of various acylations with acetylation at active TSS. Overall, these observations suggest that acylation marks could act through their competing dynamic nature rather than through stable static action and should probably not be considered individually.

In the last years new insights into the genomic distribution of diverse acylations, their role in transcriptional regulation and potential links to metabolic pathways have been gained. The combination of correlating specific acylations with gene expression states, or monitoring their enrichments at specific genomic regions by ChIP-seq, and mechanistic in vitro assays using site-specific modified chromatin can provide a powerful toolset to increase our understanding of the mechanistic and physiological functions of histone acylations.

The concept of subnuclear metabolic niches/microdomains

As described, several recent studies suggested a link between cellular metabolism and histone acylations (Egervari et al, 2020). However, we are still in an early phase concerning this integration of metabolic signals into chromatin via histone acylations, due to technical limitations and conceptual challenges that will take some time to overcome. An interesting concept within the emerging field of metaboleopigenomics are the so-called (metabolic) subnuclear chromatin niches or microdomains (Katada et al, 2012). This model is based on the notion that transcription could be regulated by the local production and accumulation of metabolites that are then utilized as cofactors by chromatin modifiers. Site-specific recruitment of metabolic enzymes to genomic regions could facilitate the creation of such nuclear microdomains with, for example, elevated acyl-CoA levels contributing to the accurate control of gene expression (Boukoursi et al, 2016). Indeed, metabolic enzymes such as ACLY, ACS52, or α-KGDH were detected in the nucleus and could potentially be involved in the local generation of acyl-CoAs (Boukoursi et al, 2016; Fang et al, 2021). It was further shown that such metabolic enzymes do not act in isolation, and that entire sections of the TCA cycle happen in the nucleus (preprint: Kalkia et al, 2020), producing locally available metabolites. How such local enrichment or pools of CoAs could be maintained is so far unknown.

Potential explanations for the generation of distinct nuclear acyl-CoA pools could be via the formation of nuclear condensates by liquid–liquid, polymer–polymer phase separation (LLPS/PPPS) or other mechanisms, which could lead to the accumulation of certain biomolecules (Erdel & Rippe, 2018; Sahari et al, 2020). LLPS describes the condensation of macromolecules into a concentrated liquid-like droplet that segregates from a less dense phase. Such a droplet could be induced by weak interactions between multivalent macromolecules that have multiple interaction domains, repeated structural domains, or intrinsically disordered regions (IDR). Phase separation could then support the dynamic creation of microdomains, in which specific proteins or molecules are concentrated and can act together (Sahari et al, 2020). Noteworthy, in vitro and in vivo evidence that LLPS is involved in the organization of the nucleus is accumulating (Larson et al, 2017; Strom et al, 2017). Histone acylations seem to be implicated in this process. Indeed, Gibson et al, (2019) showed an alteration of in vitro droplets formed by nucleosomal arrays upon their acetylation (Gibson et al, 2019). The addition of bromodomain-containing “reader” proteins to these acetylated nucleosomal arrays re-induced condensate formation. Intriguingly, these condensates were not able to fuse with droplets containing unmodified nucleosomal arrays. This finding might point toward a distinct regulation of chromatin regions marked by acylations, facilitated by LLPS (Gibson et al, 2019). However, potential effects of non-acetyl acylations on droplet formation still need to be investigated. In addition, the Kac “reader” BRD4 seems to form condensates regulating cell-identity genes in mouse embryonic stem cells, further suggesting a potential role of histone acylation in subnuclear LLPS (Sabari et al, 2018). Remarkably, a possible role of acetyl-CoA in LLPS processes was revealed recently by Houston et al, (2020). The depletion of acetyl-CoA in LLPS processes was revealed recently by Houston et al, (2020). The depletion of acetyl-CoA in LLPS resulted in nucleolus remodeling and the activation of nucleolar stress responses (Houston et al, 2020). Thus, these changes induced by acetyl-CoA depletion might indicate a potential role of CoAs in LLPS (Mitrea et al, 2016; Zhu et al, 2019).

Based on the above, we hypothesize that droplets or condensates could function to increase local concentrations of metabolic enzymes that generate acyl-CoAs, the CoAs, and chromatin-modifying enzymes, thereby contributing to the establishment of distinct chromatin microdomains (Fig 3). This could explain how different acylation/acyetlation ratios are generated at distinct chromatin regions, despite being catalyzed by the same acyltransferases.

For the validation of this hypothesis, such microdomains should be both observed in cells and subsequently disrupted and/or artificially created to test their impact on histone acylations and transcriptional regulation. A promising approach would be CRISPR-Cas9 facilitated loci-specific microdomain formation in cellulo. For example, Shin et al, (2018) used the CasDrop method that takes advantage of site-specific recruitment of dCas9 and the light-induced dimerization of target proteins to recruit proteins involved in LLPS to specific gene regions (Guntas et al, 2015; Shin et al, 2018). Similar approaches
could be utilized to recruit metabolic enzymes and acyltransferases. MS imaging could be applied to visualize and quantify histone acylations and acyl-CoAs in such metabolic microdomains (Poté et al., 2013; Thomen et al., 2020). Additionally, locally increased cofactor concentrations could be visualized by novel RNA aptamers (Paige et al., 2012). In general, improvements in super-resolution microscopy and electron microscopy will help to observe droplet formation (Mitrea et al., 2018). Recent advances in these technologies will ultimately enable us to understand and reconstruct metabolic subnuclear chromatin niches and the role of different CoAs and acylations in their formation, maintenance, and function.

Conclusion

Histone acetylation was discovered nearly 60 years ago and since then its impact on chromatin dynamics and its biological role has been extensively studied. However, the complexity and combinatorial potential of histone modifications limit a full understanding of the histone acetylome. With the identification of additional short-chain lysine modifications, the so-called histone “code” has become even more enigmatic. So far evidence for distinct functions of histone acetylation and acylation is rather limited. This raises important questions: What is the specific functional significance of these histone acylations compared with acetylation, especially considering that they often colocalize? This is particularly important considering the low nuclear levels of many acyl-CoAs (compared to acetyl-CoA) and the fact that many of the known HATs are rather poor acyltransferases. How do histone acylations contribute to the epigenetic control of transcriptional processes? And last but not least: Do they reflect cellular metabolic fluctuations caused by specific diet, fasting, development, or even disease states and help to control cellular adaption? Many studies have started to address such questions and contributed to reveal a first glimpse into the functional differences between non-acetyl acylations on histones via biochemical in vitro studies but also in vivo work. Currently, technical limitations are the major challenge that prevent us from answering some of these central questions in more detail. However, progress in the presented methods will soon pave the way for a better understanding of histone acylations in particular and metaboloepigenomics in general. Studies on the interplay between epigenetic and metabolic players in metabolic subnuclear chromatin niches might unravel how CoAs can link the metabolic state to transcriptional response via epigenetic modifications.
In need of answers

i. Which additional lysine acylations do occur on histones? What are the writers, readers and erasers? Are there any acetyltransferases that are not also acetyltransferases?

ii. How do non-enzymatic acylations contribute to chromatin function and do they functionally vary from enzymatic acylations?

iii. Do acylations have a distinct role during developmental processes or in various diseases and how do they compare with acetylation? What is their dynamic and their local abundance compared with acetylation?

iv. How does the acyl-CoA metabolism regulate gene expression programs and cell fate decisions via chromatin modifications?

v. How are metabolic niches in the nucleus formed and what are their functions? What is their role in metabolic diseases?

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Conflict of interest
The authors declare that they have no conflict of interest.

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