The Metabolic Impact on Histone Acetylation and Transcription in Ageing

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Review

The Metabolic Impact on Histone Acetylation and Transcription in Ageing

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Loss of cellular homeostasis during aging results in altered tissue functions and leads to a general decline in fitness and, ultimately, death. As animals age, the control of gene expression, which is orchestrated by multiple epigenetic factors, degenerates. In parallel, metabolic activity and mitochondrial protein acetylation levels also change. These two hallmarks of aging are effectively linked through the accumulating evidence that histone acetylation patterns are susceptible to alterations in key metabolites such as acetyl-CoA and NAD$^+$, allowing chromatin to function as a sensor of cellular metabolism. In this review we discuss experimental data supporting these connections and provide a context for the possible medical and physiological relevance.

Trends

Aging animals show global, often non-specific changes in gene expression.

Epigenetic marks such as the acetylation of histones change substantially when animals age. These changes can already be observed when animals reach midlife.

Changes in key metabolites during early aging result in changes in post-translational modifications of metabolic enzymes. This potentially leads to a transient increase in metabolic activity when animals reach midlife.

Age-dependent changes of histone acetylation are coupled to altered metabolic activity in aging animals, which could potentially influence global gene expression.

Mutations in genes that link metabolism and chromatin, such as lysine acetyl transferases (KATs), lysine deacetylases (KDACs) (sirtuins), and ATP citrate lyase (ACLY/ATPCL), have been shown to influence lifespan and the development of age-associated diseases.

The Hallmarks of Aging Are Strongly Interconnected

Several of the classical hallmarks of aging [1] correlate with epigenetic alterations that regulate transcription. Aged animals frequently show changes in gene expression [2], increased genomic instability, an erosion of telomeres, and increased cellular senescence [3]. These distinct hallmarks functionally interact with each other and affect other hallmarks such as mitochondrial dysfunction or deregulated nutrient sensing. A current challenge is to identify the functional and temporal hierarchy among these hallmarks to shed light on the connectivity of the underlying molecular processes. Such insight might allow us to identify intervention strategies that target the early stages of the aging-associated decline. Despite the frequently observed correlation between animal aging and epigenetic changes [4], the molecular mechanisms that cause these alterations are far from being understood. In this review we focus on recent data describing how acetyl-CoA metabolism and histone acetylation could lead to an age-associated disruption of the transcriptome thereby affecting animal lifespan. This intricate connection between metabolism and chromatin also influences aging-associated tissue degeneration and dysfunction. However, due to space constraints we mainly discuss its impact on lifespan.

Gene Expression Is Altered during Aging

Multiple studies have reported that alterations in the transcriptional output affect apparently unrelated genes [2,5] (Figure 1). Consistent with a stochastic loss of transcriptional control during aging, single-cell analysis of heart muscle tissue from young and old mice showed an increased cell-to-cell variation in transcription [6]. In yeast such error-prone transcription has been shown to result in proteotoxic stress and a shortened cellular lifespan [7].

Such a general change in transcription may be indicative of a general loss in chromatin structure. Aging flies show a loss of transcriptional repression of transposable elements in the adult brain, indicating a deterioration of heterochromatin [8]. Such a loss of repression of otherwise silenced

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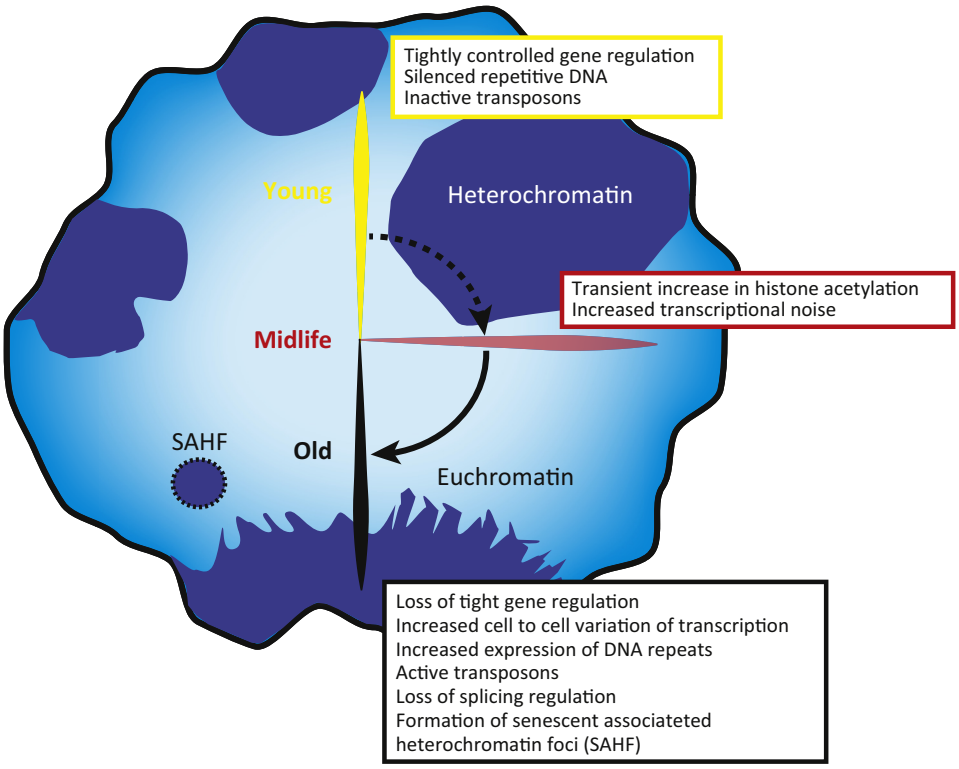

Figure 1. Chromatin Structure and Function as a Biological Clock during Aging. The transformation from young to old animal is accompanied by diverse changes in chromatin structure. Young animals display tight regulation of gene expression, maintenance of heterochromatic regions, and suppression of transposon elements. During midlife, a substantial shift in gene expression profiles is first observed. Old animals display a larger disruption of the transcriptome, reduced ability to suppress transposons, loss of heterochromatin regions, and the appearance of senescence-associated heterochromatin foci (SAHFs).

repetitive DNA chromatin has also been observed in liver and muscle tissue of old mammals [9]. A role of repressive chromatin in counteracting organismal aging is also supported by the recent finding that mutations impairing heterochromatin stability result in a premature aging phenotype [10].

The lack of repression as indicated by increased transposon activity is also observed in senescent mammalian cells [11], which have been proposed to contribute to organismal aging. This correlation between aging and cellular senescence has been suggested to occur due to impaired cellular homeostasis mediated either by a disruption of the stem cell niche or by the induction of an inflammatory response through the secretion of proinflammatory growth factors and cytokines [12]. Interestingly, senescent cells form so-called senescence-associated heterochromatin foci (SAHFs), which have been suggested to be caused by increased levels of repressive heterochromatin in these cells [13]. This is in apparent contradiction to the increased transcription of transposable elements that is usually repressed by heterochromatin [11]. A hypothesis that could possibly explain this contradiction is that the loss of repression at repetitive DNA is induced by a redistribution of known heterochromatic factors from classic heterochromatin to SAHFs in senescent cells (Figure 1). An accumulation of senescent cells in an organism is very likely to have a negative impact on lifespan as it was recently shown that clearance of senescent cells in mice is linked with a substantial increase in lifespan [14].

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In addition to showing a general impairment of transcriptional regulation, aging animals also show increases in aberrant RNA splicing [15]. Considering that chromatin structure has been shown to be involved in the regulation of alternative splicing [16,17], chromatin structure changes during aging may thus contribute to alternative splicing.

In addition to a loss in transcriptional precision that affects genes in a random manner, in some cases specific pathways also appear altered in aged animals [2,5]. A mutation in the histone demethylase UTX1, for example, delays aging in worms by targeting the insulin/IGF-1 signaling pathway [18]. Moreover, genes associated with inflammation and stress are often upregulated in old animals, while genes encoding mitochondrial and lysosome functions are reduced [2,19]. These observations underpin the complexity of the underlying changes in chromatin-transcriptional pathways during aging.

Reduction of Histone Levels and Specific Histone Modifications Contribute to Reduced Transcriptional Precision during Aging

The histone proteins both package the DNA and participate in gene expression regulation. It has been previously shown that old human cells have reduced histone synthesis [20,21] and that elevated histone expression results in lifespan extension in *Saccharomyces cerevisiae* [22]. In addition, specific histone variants such as macroH2A or H3.3 and its proteolytically processed form have been suggested to contribute to senescence [23,24].

An important aspect of histone biology is their potential to acquire a rich landscape of various post-translational modifications that affect their interaction with DNA or chromatin-associated proteins. These modifications have been shown to play a major role in the establishment and maintenance of specific gene expression profiles [25]. Besides being involved in transcriptional activation or repression, histone modifications affect transcriptional fidelity through the regulation of alternative splicing events [17] or the inhibition of initiation from cryptic promoters [26]. It is therefore conceivable that the observed loss in transcriptional precision is causally related to a change in histone modifications. A point mutant in lysine 36 of histone H3, a residue that is otherwise methylated to prevent transcription from cryptic promoters within coding regions, results in lifespan shortening in yeast [27]. Moreover, while reducing levels of H3K36 demethylases also increases lifespan in yeast and *Caenorhabditis elegans*, mutations in the H3K4 methyltransferases reduce lifespan [27,28]. Importantly, while these studies show that the loss of transcription fidelity during aging can be counteracted by modulating the activities of specific histone-modifying enzymes in yeast and worms, whether similar intervention strategies would work in mammals and in humans needs to be further tested [29]. In the following, we discuss the connection between histone acetylation, cellular metabolism, and aging in detail.

Histone Acetylation Is a Major Regulator of Transcription

Histone acetylation promotes transcription by weakening electrostatic interactions between DNA and histones and between adjacent nucleosomes within a nucleosomal fiber [30] In addition, acetylated histones form recognition sites for bromodomain-containing proteins, which are often found in transcriptional coregulators [31]. Acetylated histones are enriched over active genes and recruitment of lysine acetyltransferases (KATs), also referred to as histone acetyltransferases (HATs), increases transcription *in vitro* and *in vivo* [32,33].

According to the prevailing view, histone acetylation functions largely through the cumulative charge effects of many individual acetylation events as, at least *in vitro*, bromodomains discriminate rather weakly between individual acetylation sites [31,34–36]. However, recent studies challenge this view and indicate that some of the described redundancies are due to limited specificity of acetylation-directed antibodies (Box 1). For example, most acetylation antibodies

Box 1. Methods for Studying Histone Modifications: Challenges and Recent Technical Progress

Histone modifications are commonly monitored with antibodies. The development of antibodies with affinity towards specific histone modifications at specific amino acids ('sites') in the early 1990s revolutionized research on chromatin biology [110,111]. However, the generation of modification-specific antibodies is difficult because modifications like acetylation and methylation add only minor chemical alterations to the epitope recognized by the antibody.

Recent evidence raises considerable caution regarding the specificity of these antibodies and hence the interpretation of the data acquired using these reagents [37,112]. Antibody binding is often modulated by adjacent modifications, resulting in crossreactivities among similar peptide sequences (e.g., H3K9 and H3K27 are embedded in the same ARKS motif), and lysine methylation-directed antibodies frequently crossreact towards lower methylation states. Moreover, polyclonal antibodies can have substantial lot-to-lot variations, which can be reduced with recombinant antibodies [113]. In summary, strict antibody-validation procedures must be applied to assess the specificity of these reagents and allow biological interpretation.

Mass spectrometry offers an alternative method to study histone modifications. Mass spectrometry enables identification of new modification types and sites, the reliable quantification of extensive sets of modifications in one experiment, the identification of co-occurring modifications within the same protein sequence, and the identification of protein-binding domains with affinity towards modified peptides or nucleosomes [114,115]. However, it lacks the spatial resolution of antibody-based ChIP approaches to map histone modifications to single genomic loci. To alleviate these shortcomings, hybrid methods such as proteomics of isolated chromatin segments (PICh) or nascent chromatin capture (NCC) have been developed to quantify proteins and histone modifications at abundant genomic regions [116] or newly replicated DNA [117,118]. Moreover, the development of intracellular histone-modification-specific binders [119] and reader domain-based affinity reagents [120] offer complementary strategies to traditional antibody-based and mass spectrometry strategies. The application of these methods will help us better understand how altered histone acetylation pathways contribute to aging and aging-associated diseases, allowing the field to establish how chromatin-modification-based epigenetic changes are coupled to other hallmarks of aging [1].

show a strong polyacetylation bias and many H3 and H4 acetylation antibodies only weakly discriminate among single individual acetylation events [37].

Antibody-independent approaches suggest that acetylation sites and the combinatorial patterns they form with adjacent modifications bear more specific functions (Box 1). The binding of bromodomains to acetylated lysines, for example, is strongly modulated by adjacent modifications, including acetylation, methylation, and phosphorylation [38,39]. In line with these findings, we recently suggested that the action of many acetyltransferases may be modulated by flanking modifications. This conclusion is based on measuring the abundance changes of histone acetylation patterns by mass spectrometry after systematically depleting all known or suspected acetyltransferases that are expressed in *Drosophila melanogaster* Kc cells [40,41].

Histone Acetylation Is Altered in Aging and Aging-Related Diseases

Altered histone acetylation patterns have been observed in aging tissues and are associated with age-related maladies such as cancer, neurodegeneration, and others [4,42,43]. In many cases the changes in histone acetylation are seen on bulk histones, suggesting that they are not restricted to local and gene-specific changes in chromatin architecture but instead might potentially induce large-scale alterations of chromatin.

The reduction of H4K16 acetylation and H4K20 methylation in cells derived from a primary lymphoma has been shown to result in DNA hypomethylation of repetitive elements, which may result in higher expression of these otherwise repressed genomic regions compared with normal lymphocytes [44]. Reductions in various histone acetylation sites have also been implicated in age-associated memory impairment in mice [43]. In this case, the downregulation of histone acetylation on memory tasks such as fear conditioning in aged mice was shown to be linked to age-associated transcription disruption [45,46]. Furthermore, increasing acetylation at H4K12 using histone deacetylase (HDAC) inhibitors (HDACis) improved memory in midlife mice [45], although this observation was challenged in old rats [47]. Interestingly, higher levels of H4K12ac were described to occur physiologically in other age-related phenomena, such as mice oocyte

infertility [48] and colon cancer [49], suggesting that the effects of age-associated alterations at similar lysine acetylation sites might differ in different tissues.

Deregulation of cellular autophagy, one of the hallmarks of aging, was previously shown to be associated with increased histone H3 acetylation in aging yeast [50]. In this model, restoration of histone H3 acetylation levels enhanced the expression of autophagy-associated genes, improved autophagy, and extended yeast lifespan [51]. Similarly, a previous study showed that H4K16ac, a substrate of the deacetylase enzyme Sir2, increases during yeast replicative aging [52]. Consistent with an increased level of acetylation during aging, specific acetylation sites change in H3 and H4 when fruit flies reach midlife [53]. We surmise that further mass spectrometry-based measurements of histone modifications will increase our understanding of combinatorial epigenetic deregulation in aging and age-associated maladies.

Age-Associated Metabolic Decline Is Coupled to Changes in Acetylation

Aging has been previously associated with a general decline of mitochondrial function [54–57]. In line with this notion, a genetic mutation in the mitochondrial DNA polymerase was previously shown to cause accelerated aging in mice [58]. Supporting the functional role of metabolic decline during aging, mice with an accelerated aging phenotype show an altered serum metabolome, including reduced citrate levels, an indicator for mitochondrial activity [59]. Oxygen consumption rate is reduced in muscle fibers isolated from elderly human patients, which suggests that this tissue is also suffering decreased mitochondrial activity [60]. Nonetheless, decreasing metabolic activity via caloric restriction or targeting the activity of key metabolic regulators such as IGF-1, mTOR, and AMP kinase (AMPK) has often been associated with extending lifespan [61]. A possible explanation for such opposing results is the recent finding that transiently increased mitochondrial activity at earlier stages of aging (midlife) correlates with a shorter lifespan [53,62]. Such an increase could explain why lowered caloric intake and hence decreased metabolic activity at midlife can inhibit or delay age-associated metabolic increase.

The coupling of histone acetylation to general metabolism was previously shown to be mediated via class III HDACs (sirtuins), which are dependent on the key metabolite NAD^+ [63]. This is underscored by the observation that caloric restriction-mediated increases in healthy lifespan are partly achieved via overexpression of the Sir2 deacetylase [64]. Furthermore, the sirtuin activator resveratrol was shown to have a beneficial metabolic impact on obese humans by promoting the activity of AMPK, SIRT1, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) [65]. It is worth noting that there have been controversial discussions about the exact role of sirtuins in the aging of *S. cerevisae*, *C. elegans*, and *D. melanogaster* following rigorous control of the genetic background of the animals [66]. In particular, a concern was raised that hybrid vigor may have contributed significantly to the observed difference in lifespan in *C. elegans* and *D. melanogaster* [66,67].

Nonetheless, recent studies have shown that overexpression of SIRT1 in the brain results in an extended lifespan in mice [68]. Another study showed that whole-body SIRT6 overexpression extends lifespan in mice [69]. In addition, new studies demonstrated that caloric restriction-reduced mitochondrial protein acetylation was mediated by SIRT3 [70]. In line with these data, lower NAD^+ levels were measured in aged *C. elegans*, while restoration of the NAD^+ level resulted in an extended lifespan, which is dependent on FOXO-mediated transcription and the presence of a functional Sir2 enzyme [29]. In mice, nuclear NAD^+ levels improved the metabolic health of old mice via increased transcriptional regulation in a SIRT1-dependent manner [71], thereby further supporting a connection between sirtuin activity and aging. In summary, sirtuins have been shown to counteract multiple established aging-related pathways such as oxidative stress [72,73], protein folding stress [74–76], and genomic instability [77,78]. Considering the transient increase in protein and histone acetylation during midlife [53], the beneficial function of

sirtuins on lifespan extension might be due to sirtuin-mediated attenuation of this transient hyperacetylation during the midlife of eukaryotic organisms.

Acetyl-CoA Metabolism Affects Histone Modifications during Aging

Metabolic activity is tightly linked with chromatin remodeling via histone acetylation [79]. KATs utilize acetyl-CoA, a key metabolite primarily generated in mitochondria. Acetyl-CoA that is not metabolized via the tricarboxylic acid (TCA) cycle can be transported from the mitochondria to the cytosol by being converted to citrate; in the cytosol, citrate is cleaved back into oxaloacetate and acetyl-CoA by ATP citrate lyase (ACLY/ATPCL) [80]. Besides ACLY/ATPCL, most eukaryotes also contain a cytosolic acetyl-CoA synthase (ACS), which can contribute to cytosolic acetyl-CoA levels. However, the contribution of ACS to cytosolic acetyl-CoA levels is more important in yeast than in higher eukaryotes [81]. As acetyl-CoA can freely diffuse into the nucleus, depletion of ACLY/ATPCL in mammals results in lower histone acetylation levels [80], thereby coupling metabolism directly to histone modification [82–84]. In yeast, reduction of cytosolic acetyl-CoA via reduction in the activity of acetyl-CoA synthase (ACS2p) results in reduction of histone H3 acetylation, which is associated with increased autophagy and an extended lifespan [51]. The same study demonstrated that reduction of acetyl-CoA synthase resulted in an overall increased lifespan in *Drosophila* females. Consistent with these results, a reduction of ATPCL activity, which is physiologically increased in midlife *Drosophila* males, results in lower acetyl-CoA levels and lower acetylation of specific sites in histones H3 and H4 compared with wild-type flies of the same age [53]. Supporting the notion that the increased histone acetylation is related to the aging process, flies with lower ATPCL activity show an extended lifespan [53]. Similarly, another study demonstrated that age-related memory impairment in *Drosophila* is linked with increased pyruvate carboxylase activity [85], thereby linking acetyl-CoA levels to organismal aging.

The multiple connections between metabolic alterations and histone modifications suggest that aging-related changes in metabolism and histone modification are causally linked [51]. Mutations in genes encoding histone-modifying enzymes have been shown to affect lifespan in various model systems [4]. Several studies have demonstrated that very old animals and senescent cells show generally lower histone acetylation [86,87]. In contrast to this observation, old yeast cells show higher histone H4 acetylation levels than younger ones [52]. This apparent contradiction might be reconciled by recent data that show increased histone acetylation when animals reach midlife [51] mediated by transiently increased production of acetyl-CoA during aging [53]. Consistent with the hypothesis that such a transient increase affects aging, loss-of-function mutations of deacetylases results in shortened lifespan [88,89] whereas a mutation of the gene encoding the H4K12ac-specific KAT Chameau results in lifespan extension in *D. melanogaster* [53]. Mutations in other specific histone acetyltransferases, such as MOF [90], do not increase lifespan, suggesting a very specific effect of this KAT on aging.

In age-associated maladies such as cancer, which are characterized not only by genetic factors but also by metabolic alterations, oncogenic K-RAS causes a general increase in histone H3 and H4 acetylation via the activation of AKT1 and ACLY/ATPCL [91], which strongly supports the link between the activity of metabolic enzymes and histone acetylation. The levels of phosphorylated (active) AKT1 correlate very well with high levels of H3K9ac, H3K18ac, and H4K12ac in human tumors, further supporting a direct connection between AKT1 activity and metabolic activity. Interestingly, the activation of ACLY/ATPCL via AKT1 also connects metabolic effects to signaling cascades previously associated with organismal aging [61,92] such as IGF-1 signaling [93]. This intricate network of intracellular signaling pathways, central metabolism, and chromatin packaging further illustrates the complexity of aging in eukaryotes and explains why multiple pathways have been identified that can affect this process under laboratory conditions [1].

Box 2. Multiple Connections between Metabolism and Epigenetic Processes Can Affect Transcription and Longevity

In this review we focused on how acetyl-CoA metabolism impacts histone acetylation levels and transcription during aging. However, other epigenetic modifications are also coupled to metabolic activity and could contribute to the progress of aging and related disease. As histone and DNA methylation is also dependent on the availability of *S*-adenosylmethionine (SAM), these modifications are also likely to be connected to metabolic processes. This is evident in bees, for example, where early-life exposure to altered nutrition results in altered DNA methyltransferase Dnmt3 activity and downstream DNA methylation changes that ultimately give rise to the long-lived queen bee [121]. Altered histone or DNA methylation has been linked with various aging and longevity models [4].

Furthermore, it was recently shown that a mutation of the metabolic enzyme IDH results in the production of the metabolite 2-hydroxyglutarate (2HG), a potent inhibitor of histone demethylases, which resulted in changes of repressive histone methylations and an aberrant transcriptome in glioblastomas [122]. Thus, various metabolic signaling pathways in aging may ultimately lead to chromatin and transcription changes.

Although we chose to focus in our review on the connections of acetyl-CoA metabolism and protein/histone acetylation, other links between metabolic processes and epigenetic phenomena have also been established (Box 2). Thus, altered metabolic activity not only results in changed levels of acetyl-CoA but will also have an effect on other metabolites that potentially impact protein and histone post-translational modifications, thereby affecting lifespan or age-related diseases [18,94].

We surmise that aging results in altered metabolic activity and thus changes in downstream histone acetylation patterns as well as other epigenetic markers. Targeting the connectivity between them appears to result in increased lifespan, highlighting potential therapeutic avenues for age-related disease. It is likely that increases or decreases in the concentration of acetyl-CoA will affect the activity of cell-type-specific KATs, since these enzymes *in vitro* display K_m values in the range of the cellular acetyl-CoA concentration [95]. Alterations in acetyl-CoA metabolism could thus result in cell-type-specific age-associated transcriptome disruption [79] (Figure 2). This may account for the different transcriptional response to aging processes observed in various tissues and organisms [2]. Alternatively, but not mutually exclusively, acetylation changes mediated by metabolic changes may result in noisy transcription, which would promote progressive loss of homeostasis [53,79].

Therapeutic Application of HDACis

Targeting HDACs to restore the physiological function of many gene targets has attracted significant attention. Many studies have shown that administering HDACis could serve as a therapeutic avenue in cancer, neurodegeneration, memory impairment, anti-inflammation, and other conditions [42,96,97]. The rationale is that age-associated maladies are often characterized by lower histone acetylation (Figure 2, right panel) and thus inhibition of HDACs could correct transcriptional misregulation via increasing histone acetylation levels. Maladies associated with large transcriptomic changes could thus be targeted by using inhibitors for specific histone acetylation sites, potentially inducing changes in a large number of genes using a single drug. HDACi and bromodomain-related therapies have become an attractive area of research and several compounds are in clinical trials to treat cancer [42,98].

HDACi Therapy also Affects Non-Histone Targets

While histone acetylation has attracted considerable attention, other studies have shown that non-histone proteins are also extensively acetylated [99,100]. Interestingly, acetyl-CoA-synthesizing enzymes as well as many other enzymes involved in central metabolism were also shown to be regulated via acetylation [101]. With advances in mass spectrometry-based techniques, numerous studies have demonstrated that lysine acetylation is a widespread post-translational modification [70,102,103]. Interestingly, whereas the proportion of acetylated histone proteins is

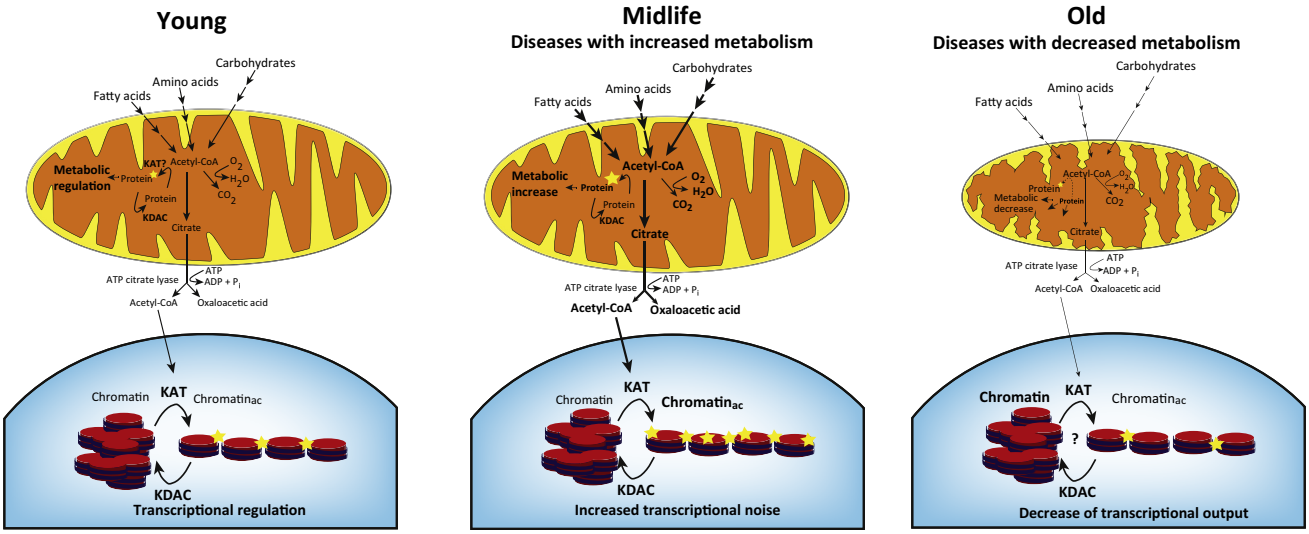

Figure 2. Metabolic Alterations in Aging Lead to Transcriptional Deregulation. Left: Young animals maintain a healthy connectivity between acetyl-CoA metabolism, protein acetylation, and histone acetylation (indicated by yellow star) resulting in regulated transcriptomes and cellular homeostasis. Middle: As animals reach midlife, small increases in metabolic activity increase the levels of acetyl-CoA and citrate. This shifts the balance towards higher levels of non-enzymatic as well as enzymatic acetylation of proteins. We suggest that increased levels of nuclear acetyl-CoA, driven by increased ATP citrate lyase (ATPCL) activity, increase the enzymatic activity of tissue-specific nuclear lysine acetyltransferases (KATs). This leads to an alteration of histone acetylation signatures and deregulation of gene expression. Such deregulation may cause cellular damage, thus increasing metabolic stress, which in turn may result in even stronger deregulation. Right: In a similar manner, lower acetyl-CoA metabolism may result in deregulated acetylomes, altered histone acetylation signatures, and a deregulated transcriptome. Such a general hypoacetylation of chromatin has, however, not yet been demonstrated in living animals.

high in cells (20–25% of histone H4 tails are acetylated in *Drosophila* and 40–50% in mice and humans), for most non-histone proteins the degree of acetylation is unknown. Currently, to detect many acetylated sites using a mass spectrometry approach, considerable pre-enrichment of the acetylated proteins must be performed, making it difficult to determine the exact proportion of acetylated isoforms [102,104]. Presently, the relevance and role of the acetylation of many non-histone proteins during aging remains unclear while we start to decipher the various roles of such acetylation events (Box 3).

Previous studies suggested that acetylation of transcription factors could impact gene expression [99] and therefore might 'rival' histone acetylation in orchestrating gene expression. This is an important aspect, since several studies have shown that HDACi treatment can impact gene expression via increasing the acetylation of transcription factors; thus, such drugs should be uniformly regarded as lysine deacetylase (KDAC) inhibitors (KDACis) [99]. We therefore need to consider alternative mechanisms through which KDACis might act as therapeutic agents; for example, by boosting metabolic activity [53] or through non-histone-dependent pathways. This may be relevant in diseases linked to metabolic alterations. We expect new studies to show revised conclusions on what is currently known about KDACi therapy [97,103] (Box 4). It is worth mentioning that recent data suggest that some KAT/KDAC effects may occur not through modulation of acetylation but rather by the modulation of acylation marks such as malonylation, glutarylation, or succinylation [105,106]. It is thus important to monitor the concentrations of all of these acyl-CoA substances. These acylations remain underexplored with regard to aging and cancer but are likely to gain more importance.

Given that metabolic enzymes are partially regulated by acetylation and histone acetylation regulates transcription, the overall cellular changes that are affected by KDACi treatment should be revisited [94,103,107,108]. Such analyses, along with continuing improvements in advanced

Box 3. A Large Number of Non-Histone Proteins Are Acetylated

Recent developments in mass spectrometry technology have revealed thousands of non-histone acetylation sites following enrichment of acetylated lysines using anti-acetyl-lysine antibodies [70,102,103]. Lysine acetylation has an impact on multiple cellular pathways ranging from metabolism to gene expression [100]. Various metabolic enzymes involved with acetyl-CoA metabolism are themselves acetylated, which results in the generation of multiple but often hypothetical autoregulatory loops [101,123]. Interestingly, many acetylated proteins are detected in mitochondria, where the evidence for specific lysine acetyltransferases is sparse. It has therefore been suggested that a large fraction of mitochondrial acetylation might be the result of non-enzymatic acetylation [124]. Consistent with the observation that most of the known acetyltransferases are found in the nucleus, a substantial fraction of acetylated proteins are also found in the nucleus, where acetylation regulates chromatin packaging and transcription [99,107]. Besides its effect on metabolic regulation and gene expression, protein acetylation has also been implicated in the regulation of DNA repair [108], autophagy [125], and protein stability [126,127].

Intracellular acetyl-CoA levels are known to change dynamically in response to multiple physiological stimuli or pathological processes [94]. It is therefore likely that future studies will link specific protein acetylations to the progression of specific diseases, thereby unraveling novel drug targets for protein acetylation.

Box 4. Revisiting the Mechanism of KDACis in Light of the Acetylome

The possible impact of KDACis on cellular metabolic activity has remained underexplored, despite their known effect on the acetylation of metabolic enzymes [102,103,128]. Administration of the broad KDACi sodium butyrate or trichostatin A to whole fly-head tissue results in a transient and rapid increase of oxygen consumption, underscoring the effect of these drugs on metabolism [53]. Considering the dependency of histone acetylation on acetyl-CoA levels [80], it is likely that KDACis induce histone acetylation not only via direct inhibition of histone deacetylases but also because of their effects on metabolism [53,109]. Furthermore, it is possible that KDACi-mediated epigenetic therapies exert their effects via alternative and non-epigenetic pathways; for example, by affecting the acetylation of non-histone and non-chromatin proteins. It is therefore possible that chronic administration of a KDACi could result in unwanted metabolic stress, with substantial side effects [53]. Thus, it may be necessary to reinvestigate many KDACi-based treatments with regard to their *in vivo* targets to enable better risk assessment and to potentially develop combination therapies that also target metabolic pathways.

Outstanding Questions

What are the molecular mechanisms that drive metabolic alterations during early ageing? Are changes in post-translational modifications of histones and metabolic enzymes a cause or a consequence of increased metabolic activity?

Does caloric restriction and lower mitochondrial activity lead to an extension of lifespan by counteracting increased metabolic activity during midlife?

Can specific histone modification patterns be indicators of molecular ageing?

Does a general deterioration of tight gene regulation lead to cellular aging or are specific signaling pathways involved in the aging process?

How much does non-histone acetylation contribute to the aging process?

What are the non-histone targets of specific KDACis? Is the therapeutic effect of KDACis in cancer, neurodegeneration, and other maladies due to modulation of metabolism rather than regulation of gene expression?

Are small-molecule modulators of metabolic enzymes potential drug candidates to alleviate age-related disease?

mass spectrometry-based and computational tools to precisely measure post-translational modifications of histones and non-histone proteins, will lead to an improved characterization of metabolic–epigenetic deregulation in aging and disease. This should lead to the development of more specific drugs while limiting their side effects.

Concluding Remarks

There is growing evidence that chromatin remodeling via histone acetylation is tightly linked to metabolic activity [5,109]. It is evident that age-associated alterations in metabolic activity change the concentration of acetyl-CoA and of citrate and therefore will affect cytosolic levels of acetyl-CoA. Changes in this metabolite will immediately affect other metabolic processes such as the synthesis of fatty acids, which may have an effect on multiple aspects of cellular physiology. However, changes in acetyl-CoA can also impact levels of histone acetylation and disrupt the regulation of transcription as animals age or develop aging-related diseases [51,53]. It is likely that distinct KAT and KDAC enzymes possess specific affinities for their cofactors acetyl-CoA and NAD^+, respectively, and may thus respond differently to age-associated alterations in cofactor concentrations [2]. Moreover, additional connections between metabolic activity and other epigenetics marks (methylation, phosphorylation, butyrylation, and others) may also contribute to age-associated transcriptional regulation. We expect that further experiments uncovering even closer connections between metabolic changes in aging and the deregulation of gene expression will offer new strategies to increase healthy lifespan and prevent related diseases [61].

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