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Chromatin: A Capacitor of Acetate for Integrated Regulation of Gene Expression and Cell Physiology

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Summary

Cancer tissues with lower global levels of histone acetylation display significantly increased rate of tumor recurrence or cancer-related mortality. The function global regulation of histone acetylation serves for the cell or how lower levels of histone acetylation may contribute to a more aggressive cancer phenotype has been unclear. Chromatin and histone modifications are currently thought to regulate only DNA-based processes. However, recent findings have revealed a novel function for global histone acetylation in direct regulation of cellular physiology. I will discuss how chromatin, by regulating the cellular flux of acetate, may integrate control of cellular physiologic state with gene expression and help explain the observations in cancer tissues.

Introduction

Local regulation and function of histone modifications: the current paradigm

Understanding the distributions of histone modifications across the genome in a variety of biological settings has become a priority for the epigenetic community and has led to significant investment in large-scale mapping efforts such as the NIH Epigenome Roadmap [1]. These maps are used to infer the functions of histone modifications by comparing the location of modified histones to other genomic landmarks or to nearby DNA-based processes such as transcription. At specific genomic loci, single or combinations of histone modifications may alter the local chromatin structure and/or serve as binding sites for recruitment of protein complexes with regulatory activities to the vicinity of DNA. This approach is based on what I call a “Positional Indexing” paradigm which assumes that histone modifications are regulated locally and mostly independently of other regions of the genome and function mainly to regulate proximate DNA-based events. While Positional Indexing has provided a useful intellectual framework for understanding the functional relevance, causally or correlationally, of histone modifications to various genomic

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processes, this paradigm is too limited to explain the alterations in global levels of histone modifications observed in cancer as discussed below.

The biological relevance of global regulation of histone modifications

Individual cells or tissues display dramatic differences in the total levels of histone modifications contained within cells. Such cell to cell or tissue to tissue heterogeneity has been demonstrated by western blotting or immunohistochemical analysis of various cell types or tissues. For example, cells of skeletal muscle, heart and stomach contain much lower levels of histone acetylation compared to other tissues such as lung or colon [2]. But whether such regulation was biologically meaningful had remained obscure until our laboratory showed that the differences in global levels of histone acetylation and methylation of specific residues are prognostic of clinical outcome in cancer patients. Specifically, increased prevalence of cells with lower global levels of histone acetylation and/or methylation was associated with increased risk of tumor recurrence in prostate cancer [3]. The prognostic utility of global histone modification patterns is not specific to prostate cancer but has been extended by several laboratories to multiple other cancer types, revealing a surprisingly common feature among adenocarcinomas [4–7]. In most cases, lower global levels of histone acetylation and methylation has been associated with more clinically aggressive cancers of several organs, regardless of tissue of origin, and poor patient outcome such as decreased survival probability (Fig. 1) [8,9].

This prognostic link has underscored the biological relevance of global regulation of histone modifications. Maintenance of global histone acetylation requires a pool of ac-CoA generated from glucose, acetate or other carbon sources as well as enzymes involved in ac-CoA synthesis such as ATP citrate lyase and ac-CoA synthetase [10–12]. But it has been unclear what cellular processes might be affected by regulation of histone modifications at a global scale. Even more challenging has been the difficulty to formulate coherent and testable hypotheses within the Positional Indexing paradigm that would explain several observations made from analyses of global histone modifications in primary cancer tissues. First, it is hard to envision how lower levels of histone modifications would be associated with an aggressive phenotype in cancers that are derived from distinct tissue types with different gene expression patterns and mutational profiles. Second, different histone modifications are established by different sets of enzymes, yet most modifications show similar associations with cancer aggressiveness, requiring the balance of addition and removal of the modifications be tipped consistently toward the latter in aggressive cancers. Third, and most curious, the normal cells such as stromal fibroblasts within cancer tissues, show similar levels of global histone modifications as those of neighboring cancer cells—when cancer cells exhibit lower global levels of histone modifications, so too the normal cells in the same cancer tissue. These findings suggest that despite their prognostic relationship, the alterations in global levels of histone modifications are not cancer-cell specific, and may operate in both normal and cancer cells within a given tissue. An outstanding issue from the cancer studies has been whether the low global histone modification levels represent an active or a static epigenetic state. Do these cancers contain little histone acetyl- or methyltransferase activities resulting in hypo-modified histones? Or

is the rate of deacetylation/demethylation higher than acetylation/methylation and thus histones only *appear* hypo-modified?

Global regulation of histone acetylation is linked to the physiologic state of the cell

Addition or removal of histone modifications involves metabolites that are central to many catabolic and anabolic reactions such as acetyl coenzyme A (ac-CoA) and acetate in the case of histone acetylation and deacetylation, respectively. Acetate as a two carbon unit is a building block for biosynthesis of cellular macromolecules such as fatty acids but is also used as a post-translational modification. In the case of histones, not only they are heavily acetylated but the acetyl groups are turned over rapidly within minutes when measured by radioactive acetate labeling [13–15] (measurements using labeled glucose yield slower turnover rates [16,17]). Histone acetylation turnover rates are so far measured only in nutritionally replete and physiologically balanced conditions; but the rates may be faster or slower in different metabolic and physiological conditions. The turnover of histone acetylation is essentially converting ac-CoA to acetate anions and free CoA as histones are rapidly and continuously acetylated and deacetylated. The sheer scale and the short transit time of acetate molecules on histones could constitute a substantial fraction of cellular carbon pool that passes through chromatin. Regulation of the speed of acetate flux through chromatin—via modulation of the balance of acetylation and deacetylation reactions—could therefore determine the global levels of histone modifications at any given time; and also serve to regulate the availability of acetate anions as they get liberated from histones. Such regulation could be made responsive to certain biological cues such as metabolic or physiologic events that rely on supply of acetate.

Following this premise, our laboratory systematically analyzed the various components of a typical cell culture media to determine whether metabolic or physiologic perturbations affect global levels of histone modifications [18]. In none of the experimental conditions did we observe a significant change in global levels of histone methylation. I therefore will limit the discussion henceforth to histone acetylation. Of all the available carbon sources in the media, glucose, glutamine or pyruvate alone was sufficient in maintaining the global levels of histone acetylation at a similar level to that found when cells (HeLa, MDA-MB-231 and primary lung fibroblasts) were cultured in complete media. Removal of these carbon sources resulted in a gradual and stable loss of histone acetylation globally. But surprisingly addition of minimal amounts of glucose or glutamine at concentrations significantly below what is normally required for sustaining cell division did in fact restore normal levels of histone acetylation. Addition of all three nutrients had no additive or synergistic effects on global histone acetylation levels. So, while glucose, glutamine or pyruvate is required for histone acetylation, it is unlikely that availability of these carbon sources is related to the global alterations in global histone acetylation observed in cancer tissues.

In analyzing various physiological parameters, we made the surprising discovery that global acetylation and deacetylation of histones is in flux with exogenous acetate and protons and the direction of this flux is sensitive to intracellular pH (pH_i) [18]. Cells must constantly buffer their pH_i against extreme acidity or alkalinity—due to pH changes in microenvironment or intracellular metabolic activity—to function properly and have

therefore acquired multiple mechanisms to maintain their pH_i. These mechanisms include sodium/proton and chloride/bicarbonate exchangers, proton pump ATPases as well as the monocarboxylate transporters (MCTs) [19,20]. MCTs in particular have been shown to function mainly when pH_i drops below 7 [20–22]. Acetate and other small organic acids are primarily transported in and out of the cell through the MCTs [23]. Interestingly, MCT1-4 proteins are obligate proton symporters, coupling the transport of small fatty acids such as acetate to protons [23]. In rapidly growing cells in typical DMEM, the pH_i is slightly alkaline (pH~7.4) and although acetate is continually exchanging with the outside environment, the net flow of acetate is toward inside the cell with high levels of global histone acetylation. As pH_i becomes more acidic especially as it goes below pH_i~7.0, the net flow of acetate reverses and becomes outward. At the same time histones are increasingly deacetylated leading to appearance of globally hypoacetylated chromatin. Increased and continual deacetylation of histones liberates acetate anions that are then exported out of the cell along with protons. The net effect of global and dynamic histone deacetylation and export of acetate and protons out of the cell is decreased proton concentration inside the cell and buffering the cell against further acidification [18]. Thus by regulating the availability of acetate for proton transport, chromatin in effect regulates the cellular proton load, and hence, pH_i (Fig. 2). Importantly, we ruled out a connection between global acetylation levels and the degree of glycolytic phenotype (i.e., the Warburg effect) so long as the cells had similar intracellular pH levels (unpublished data).

These findings have now provided a plausible explanation for why cancers with lower histone acetylation levels are more aggressive. It is possible that these cancers are experiencing an acidic microenvironment and are continuously deacetylating their histones to generate acetate anions for proton export to maintain a viable pH_i. It is not uncommon for tumors to have low interstitial pH and in fact measured pH of cancer tissues in vivo is commonly in the range of 6.4–7.5 [24–26] and in certain cases as low as pH 6 [27]. Such acidic conditions select for more resilient cells that are resistant to chemotherapy and/or radiation [28–31], potentially explaining the association of low histone acetylation with aggressive cancers, regardless of the tissue of origin. Even normal stromal cells within acidic cancer tissues or normal cells with low pH_i such as skeletal muscles (pH_i~6.0 [32]) could use histone acetylation to prevent even further intracellular acidification.

Regulation of intracellular pH by histone acetylation is also conserved in the budding yeast which also globally deacetylates its histones in response to acidity. Interestingly, a screen of yeast gene knockout collection for mutants with low global histone acetylation levels identified several members of the Vacuolar-type H^+-ATPase (V-ATPase) [33]. This proton pump uses the energy of ATP hydrolysis to transport protons across intracellular and plasma membranes into the vacuole or to the extracellular space. Disruption of this enzyme complex leads to acidification of cytoplasmic and nuclear compartments due to insufficient proton transport into the vacuole or out of the cell, potentially obliging the cell to increase histone deacetylation to generate acetate anions for proton export. This could explain why yeast cells lacking the V-ATPase genes have low global levels of histone acetylation.

Relating the 'global' effects to the molecular distribution of histone acetylation

The term 'global' is used to describe broad changes in the levels histone acetylation at the whole cell level. However, this term is too simplistic and naively implies that all histone acetylation sites across the genome are functionally equivalent. For instance, at the molecular level, vast regions of the genome are deacetylated on histone H4 lysine 16 (H4K16) at low pH but a limited and unique number of chromosomal domains are newly acetylated [18]. And not all hypoacetylated regions are similar in nature. Some hypoacetylated regions are dynamically deacetylated as they regain acetylation when treated with HDAC inhibitors; while other hypoacetylated regions are subject to one-off deacetylation events and remain deacetylated even in presence of HDAC inhibitors (unpublished data). So, the global changes in histone modification levels are principally an indication of an 'extreme makeover' of the epigenome: extensive redistribution at the genomic level that is associated with considerable changes in the total number of modified loci.

Since most histone acetylation peaks are by and large at promoters, enhancers or gene regulatory regions, the response of histone acetylation to pH variation could be integrated with control of gene expression [18]. At this point, there is not adequate information on which HDACs may be involved in response to pH, how they may sense changes in pH or modulate their enzymatic activities. More information on the distributions of several histone acetylation sites at different pH levels are also needed to delineate how changes in levels and distributions of the individual histone lysines correlate with the transcriptional adaptations that occur when pH_i is altered. But it is tempting to speculate that pH regulation or proton dynamics may be an element in the logic by which certain genes or regions in the genome are selected for targeting by a given HDAC. For example, the *S. cerevisiae* major HDAC RPD3 (for *R*educed *P*otassium *D*ependency 3) is involved in regulation of phosphate and potassium uptake genes as well as genes targeted by the transcription factor Opi1, a negative regulator of phospholipid biosynthesis [34–36]. The biology of these Rpd3 target genes are all linked to dynamics of proton regulation [37–39].

Chromatin as a carbon capacitor

We have known for decades that metabolic pathways provide metabolites and co-factors for epigenetic enzymes to modify chromatin for regulation of gene expression. The response of chromatin to pH variation has generated an expanded paradigm, one in which chromatin is not simply the recipient of metabolic inputs but may provide regulatory feedback through regulation of gene expression *and* provision of metabolites back to metabolic or physiologic pathways (Fig. 3). In the case of acetylation, histones may function as a 'way station' in the flux of cellular acetate and together with the use of the numerous histone acetyltransferase and deacetylase enzymes, impart chromatin with the capability to function as a capacitor for storage and release of acetyl groups as dictated by cellular metabolic or physiologic needs.

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* of special interest

** of outstanding interest

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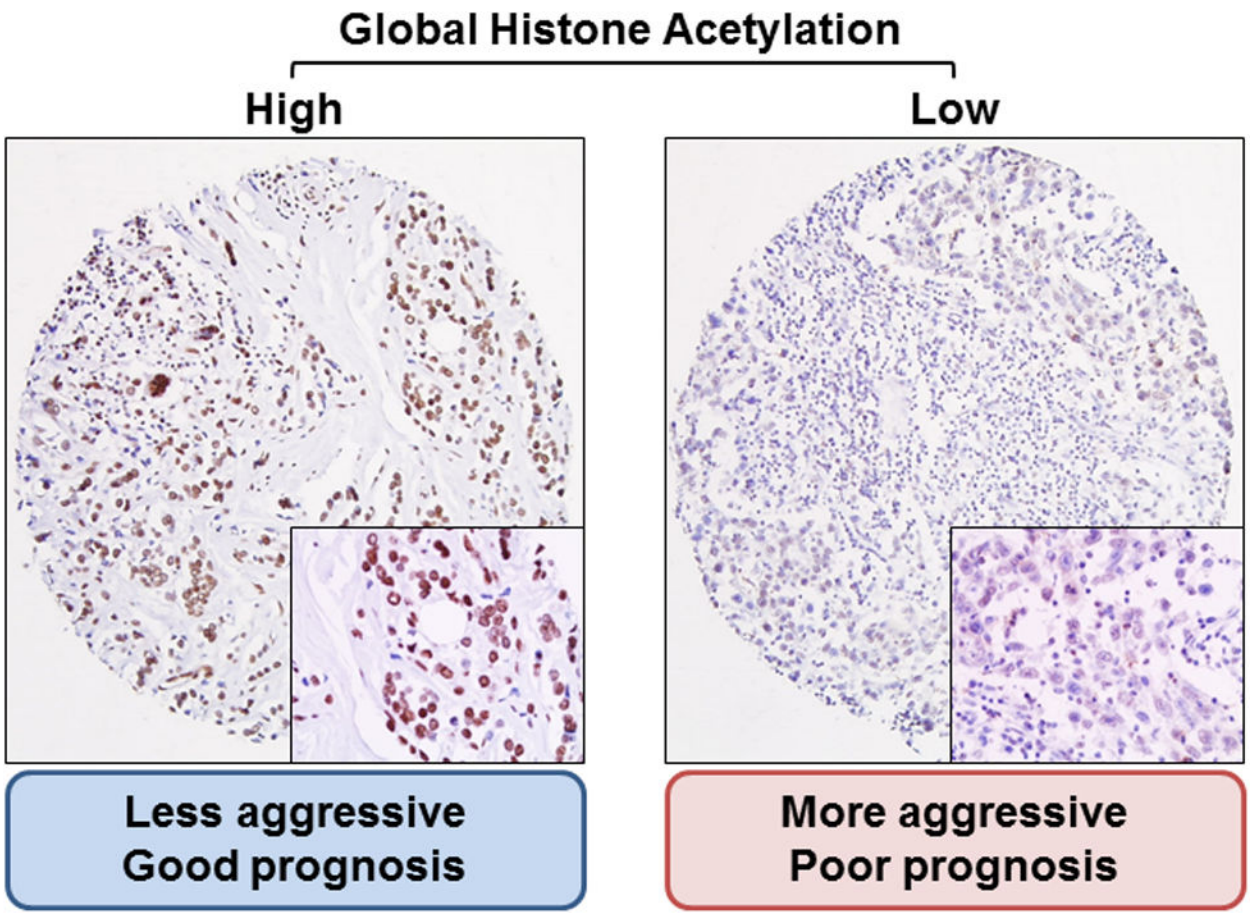

Figure 1. Global histone modification levels as cancer prognostic markers

Immunohistochemical staining of primary cancer tissues with an anti-histone 3 lysine 18 acetylation (H3K18ac) antibody shows cellular heterogeneity in global levels of H3K18ac. Cells with high and low levels of histone acetylation are stained brown and blue respectively. Generally, lower levels of most histone acetylation and methylation sites are predictive of poorer prognosis. Note that the normal cells within the stroma of the cancer tissues have similar global levels of histone acetylation as cancer cells. Based on the model proposed in this article (see Fig. 2), cancer tissues with low global histone acetylation levels may be experiencing an acidic environment and are actively deacetylating their histones to liberate acetate anions for proton export.

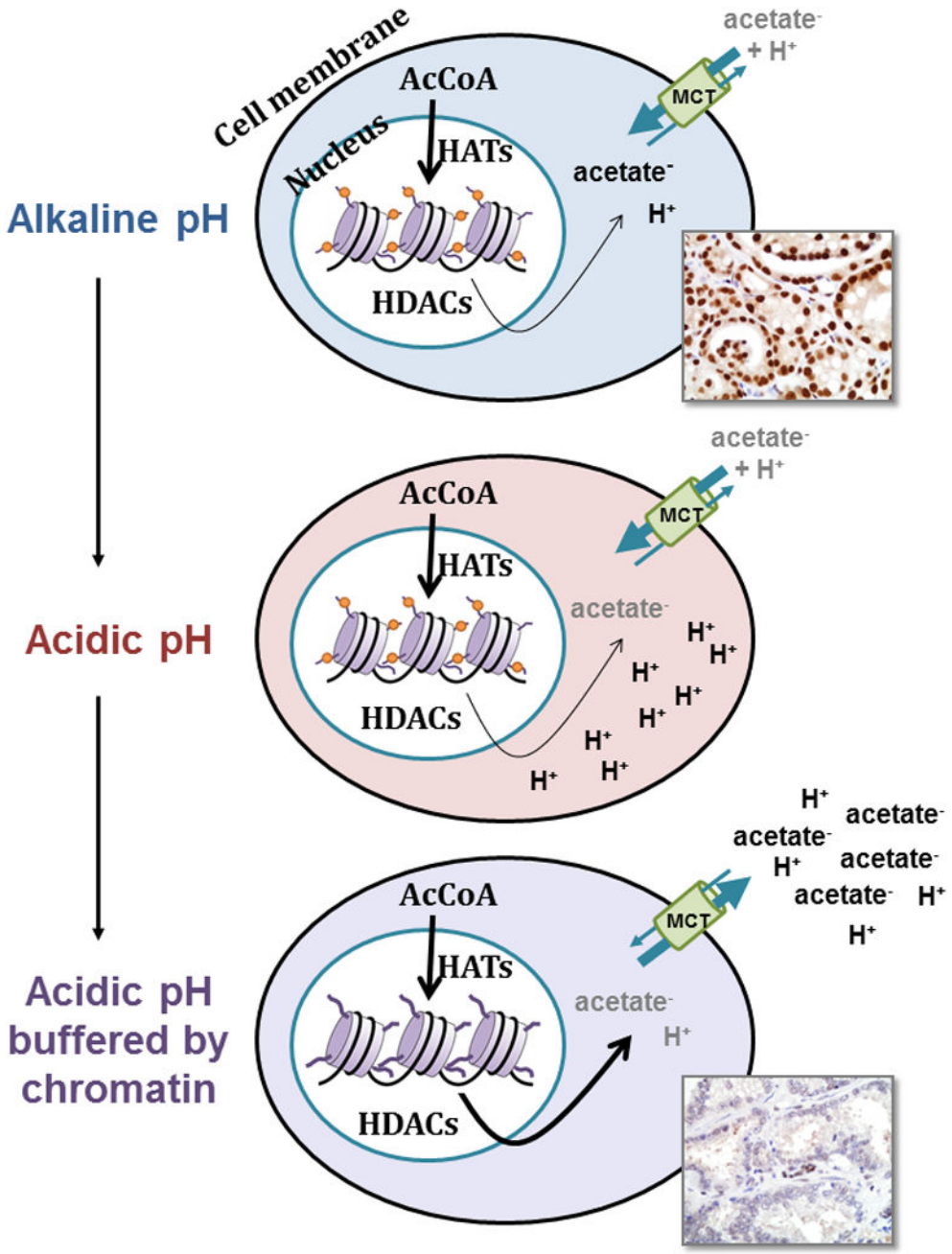

Figure 2. Chromatin as a rheostat for regulation of intracellular pH

A proposed model for how dynamic and global histone acetylation and deacetylation together with the cellular flux of acetate and protons may regulate intracellular pH. Although intracellular acetate is continually exchanging with exogenous acetate, in alkaline pH the net flow of acetate and protons is inward, histone acetylation is favored and histones appear hyper-acetylated (upper panel). Cancers with high global acetylation levels may be in such a condition (inset). Due to increased acidity in the extracellular environment or other cell-intrinsic processes, protons may accumulate in the cell, leading to decreased intracellular pH (middle panel). To buffer the cell against further acidification, histone

deacetylation is favored—in the face of continual acetylation—with the net flow of acetate anions and protons toward the outside of the cell which decreases the intracellular proton load (lower panel). In these acidic conditions, histones *appear* hypo-acetylated, a condition which may explain cancers with low global levels of histone acetylation (inset). This model proposes that chromatin functions as a capacitor for acetate flux, regulating the availability acetate anions through the counteracting actions of histone acetyltransferases (HATs) and deacetylases (HDACs). A prediction of this model that HDAC inhibitors may disrupt the cellular pH buffering capacity by diminishing the availability of acetate for proton export has been borne out by experiments [18]. (MCT: monocarboxylate transporter. The orange circles represent acetylation of histone N-termini.)

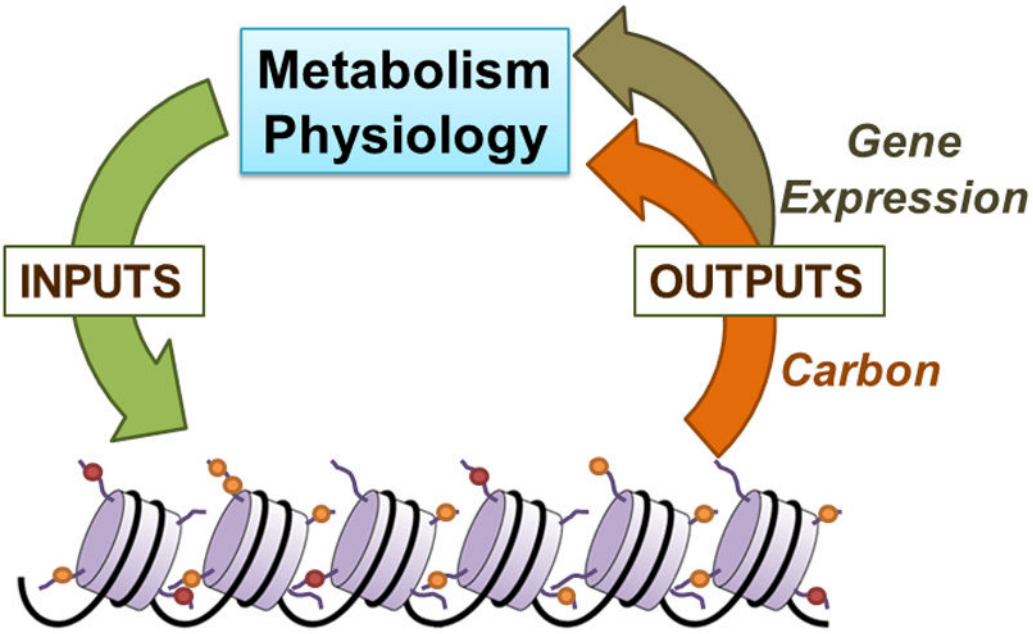

Figure 3. Chromatin as a regulator of carbon flux and gene expression

A model for how chromatin may be integrated into the broader metabolic and physiologic networks of the cell. Chromatin may receive inputs from various metabolo-physiologic pathways. These inputs may be in the forms of active metabolites and co-factors for epigenetic enzymes but also relay information about the state of the cell such as pH. Chromatin in turn responds by changing the patterns of gene expression and/or provision of carbon in the form of acetate to modulate cellular metabolism and physiology. Similar concepts may apply to histone methylation and other modifications. (The orange and red circles represent acetylation and methylation of histone N-termini, respectively.)