Epigenetics and Metabolism

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Abstract: The molecular signatures of epigenetic regulation and chromatin architectures are fundamental to genetically determined biological processes. Covalent and post-translational chemical modification of the chromatin template can sensitize the genome to changing environmental conditions to establish diverse functional states. Recent interest and research focus surrounds the direct connections between metabolism and chromatin dynamics, which now represents an important conceptual challenge to explain many aspects of metabolic dysfunction. Several components of the epigenetic machinery require intermediates of cellular metabolism for enzymatic function. Furthermore, changes to intracellular metabolism can alter the expression of specific histone methyltransferases and acetyltransferases conferring widespread variations in epigenetic modification patterns. Specific epigenetic influences of dietary glucose and lipid consumption, as well as undernutrition, are observed across numerous organs and pathways associated with metabolism. Studies have started to define the chromatin-dependent mechanisms underlying persistent and pathophysiological changes induced by altered metabolism. Importantly, numerous recent studies demonstrate that gene regulation underlying phenotypic determinants of adult metabolic health is influenced by maternal and early postnatal diet. These emerging concepts open new perspectives to combat the rising global epidemic of metabolic disorders. (Circ Res. 2015;116:715-736. DOI: 10.1161/CIRCRESAHA.116.303936.)

Key Words: chromatin ■ diet ■ epigenetics ■ glucose ■ histones ■ metabolism ■ methylation

Living organisms and individual cells manage environmental variations with rapid and stable alterations in gene expression. The key to this adaptive agency is the chromatin polymer, a dynamic constituent assembly of DNA and various nucleoproteins that spatially regulates transcriptional competency by structural adaptation and genome compartmentalization. Covalent epigenetic modification imparting functional modulation and structural chromatin reorganization that potentiate a wide range of adaptive phenotypes are increasingly examined in human health and disease. The immediate cellular environment provides the contextual determinants of epigenetic chromatin architecture. Evidence for the integration of subcellular, global, and external metabolic information into this intricate system of gene regulation has recently begun to emerge.

Chromatin modification as a stable system of metabolic information can be observed at regulatory and coding elements of many genes implicated in metabolism. Postreplicative methylation of DNA predominantly at the 5-carbon ring of cytosine...
nucleotides (5-methylcytosine [5mC]) adjacent to guanine residues (CpG) at gene promoters is associated with gene suppression and corresponds most closely to the etiological interpretation of epigenetics. Mechanistically, 5mC is interpreted by regulatory proteins and the recruitment of chromatin remodeling complexes to establish transcriptionally repressive chromatin.\(^1\) Equally important are the wealth of covalent post-translational modifications (PTMs) dynamically written to and erased from N-terminal tails of chromatinized histone proteins by specialized enzymes and complexes. Here, the methyl modification also occupies a prominent role in gene regulation when assigned to arginine and lysine residues by histone methyltransferases, and the relationship to transcription is primarily dependent on the site of histone tail methylation. For example, methylation at lysines 9 and 36 of H3 histones associates with gene repression and activation, respectively. Furthermore, different degrees of methylation (arginine residues can be mono- and asymmetrically or symmetrically di-methylated, and lysine residues mono-, di-, or trimethylated) are the products of specific enzymes, differentially distributed across chromatin, and ascribed distinct functional roles in gene regulation. For instance, transcriptional activity is positively correlated with the degree of H3-lysine-4 trimethylation (H3K4me3) at the 5′ end of actively transcribed genes,\(^3,3\) whereas the monomethylated form of this modification (H3-lysine-4 monomethylation) is not restricted to promoter regions but is also strongly enriched at distal regulatory elements.\(^4\) Although H3K4me3 has been shown to facilitate the recruitment of specific complexes associated with chromatin remodeling,\(^5\) the function of H3-lysine-4 monomethylation is less clear. In addition to its role in discrete gene-activating events,\(^6\) H3-lysine-4 monomethylation was recently found to mark the promoters of a subset of repressed genes and spatially restrict the occupancy of H3K4me3 readers such as ING1.\(^7\) Countering the activities of histone methyl writers are methyl erasers whose enzymatic activity could establish specific modifications such as H3-lysine-4 monomethylation by reversing higher methylated states.\(^8\) On the other hand, site-specific acetylation instructed by opposing enzymatic functions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) almost exclusively marks chromatin for transcriptional competency (Figure 1). These and other PTMs such as ADP-ribosylation, phosphorylation, and sumoylation, as well as recently described interactions with noncoding RNA transcripts,\(^9\) collaboratively generate the dynamic epigenomic landscape by extensive cross talk with transcription factor networks to contextualize gene activity. A recently emerged and perhaps underappreciated link centers on how the chromatin-modifying activities of epigenetic enzymes are influenced by intracellular concentrations of essential substrate cofactors that are also intermediates of major metabolic pathways.\(^10\)

The rising global epidemic of obesity and metabolic disorder underscores the current challenge of mapping the metabolically responsive epigenome. Studies have started to define molecular mechanisms linking fat and glucose metabolism to nuclear transcription, highlighting influential epigenetic regulatory determinants. Moreover, mounting evidence indicates that altered metabolism contributes to disease by distorting epigenetic regulation. Building on important epidemiological and clinical observations of persistent perturbations converging on gene deregulation in metabolic disease,\(^11–13\) recent experimental findings suggest that the reputed stability of gene-activating epigenetic changes may confer future cell memories. Well-known examples of long-term persistence include the ongoing vascular injury experienced by patients with diabetes mellitus many years after effective glycemic control is achieved.\(^14\) A similar and more recently emerged connection between the nutritional environment of the developing fetus and chromatin changes underlying pathological gene expression linked with adult disease is rapidly gaining momentum.\(^15\) Although not exhaustive, this review discusses the most recent key findings that link cellular metabolism with chromatin-dependent gene changes. Emphasis is placed on observations that define this nexus in metabolically important organs such as the pancreas and liver, with pronounced implications for human metabolic health. Furthermore, dietary factors conferring epigenetic changes associated with disease in development and adulthood are reviewed in context of the increasing prevalence of human metabolic dysfunction.

**Metabolite Cofactors of the Epigenetic Machinery**

By serving as essential cofactors for most chromatin-modifying enzymes, important intermediates of cell metabolism and dietary intake allow the integration of metabolic information and transcriptional control (Figure 2). Fluctuating metabolite concentrations are therefore proposed to provide signaling cues for continual adjustment of gene expression by modulating the epigenome to influence chromatin dynamics. In addition to overall metabolic changes, the epigenome could be further shaped by the subcellular localization of metabolite synthesis and distributional variations in concentration.\(^16\) Numerous epigenetic enzymes may compete for the same cofactor to modify distinct substrates, and recent reports describe the coupling of cofactor synthesis and histone modification reactions to coregulate gene expression.\(^17,18\) Additional biochemical evidence suggests that energy metabolite concentration could affect PTM of the chromatin-modifying machinery itself, in turn regulating enzymatic activity, stability, and chromatin binding capacity associated with gene expression.
Metabolism and the Methylome

As the universal substrate for methyltransferase reactions on DNA as well as arginine and lysine residues of histones and other proteins, S-adenosyl methionine (SAM) provides substrate for some of the most prevalent PTMs. By contrast, the S-adenosyl homocysteine biproduct of these reactions is a potent methyltransferase inhibitor. Diverse biological outcomes are conferred by distinct methyl events on a variety of substrates and altered chromatin methylation patterns are extensively implicated in metabolic gene activation and repression. Unlike the aforementioned intermediates of energy pathways, SAM is derived from dietary methyl group consumption and 1-carbon metabolism primarily involving methionine, folate, and choline. The importance of dietary methyl donors to epigenetic regulation has been examined in rodent models as well as in humans, and the effects of maternal dietary intake are a key focus of recent studies.

How the enzymes responsible for individual modification patterns interrelate has been the subject of recent studies. For example, competition for available SAM may regulate the contrasting events associated with transcriptionally permissive methyl-H3K4 modifications and repressive methyl-H3K9 or methyl-CpG modifications. Table 1 describes mammalian methyltransferases that might compete for bioavailable SAM to modify chromatin. In addition, the importance of post-translational methylation is emerging for a growing list of nonhistone substrates including transcription factors and many important components of the methylation machinery such as SUV39H1 and DNMT1 are themselves activated and destabilized respectively by methylation. A deeper comprehension of methyltransferase kinetics will undoubtedly enhance our appreciation for the complexities of this prevalent and highly ordered system of chemical modification.

Connections between metabolic cofactors and enzymes associated with the removal of epigenetic methyl modifications are also beginning to surface. Until recently, 5mC was the only epigenetic mark known to occur on DNA. Important discoveries of 5-hydroxymethylcytosine and the ten-eleven translocation (TET) family of dioxygenases (TET1/2/3) that generate this base by oxidation of 5mC in mammalian cells have inspired strong interest in transitions between methylated and unmethylated DNA. In addition, oxidation of methyl-H3K4 modifications and repressive methyl-H3K9 or methyl-CpG modifications.
5-hydroxymethylcytosine by TETs to 5-formylcytosine and 5-carboxylcytosine further expands the list of known DNA base modifications. Although precise mechanisms are yet to be completely defined, recent data demonstrate the importance of TET activity in repair-associated and targeted mechanisms of DNA demethylation. The potential for TETs to regulate diverse physiological functions includes metabolic signaling because TET-mediated oxidation of 5mC requires the tricarboxylic acid (TCA) cycle metabolite α-ketoglutarate, and is inhibited by 2-hydroxyglutarate. In addition, Jumonji C domain containing (JmjC), LSD1. The alternative route of glucose metabolism through the hexosamine biosynthetic pathway (HBP) generates uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) that is utilized by O-GlcNAc transferase (OGT) for histone GlcNAcylation. Metabolic cofactors for chromatin-modifying enzymes are shown in red. Several of these reactions directly associated with energy metabolism also occur in the cytoplasm.

Figure 2. Intermediates of energy metabolism are cofactors for chromatin modifications. The tricarboxylic acid (TCA) cycle links energy pathways with epigenetic chromatin modifications (blue). Glycolysis and β-oxidation generate acetyl-CoA that feeds into the TCA cycle and also provides substrate for histone acetyltransferases (HATs). Nicotinamide adenine dinucleotide (NAD+) is required for the histone-modifying activities of sirtuin histone deacetylases (HDACs; histone deacetylation) as well as ADP-ribosyltransferases (ARTs). α-Ketoglutarate and flavin adenine dinucleotide (FAD) are cofactors for DNA (TETs) and histone demethylases (Jumonji C domain containing [JmjC], LSD1). The alternative route of glucose metabolism through the hexosamine biosynthetic pathway (HBP) generates uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) that is utilized by O-GlcNAc transferase (OGT) for histone GlcNAcylation. Metabolic cofactors for chromatin-modifying enzymes are shown in red. Several of these reactions directly associated with energy metabolism also occur in the cytoplasm.
to directly facilitate mitochondrial gene expression.\textsuperscript{60,61} More recently, the DNMT3a methyltransferase was observed in the mitochondria of mouse and human neurons.\textsuperscript{62} Similarly, binding of a mitochondrial isoform of DNMT1 to mtDNA, as well as enrichment for both 5mc and 5hmC in human cancer cells and mouse embryonic fibroblasts,\textsuperscript{63} strongly implicates epigenetic modulation in mammalian mitochondrial gene transcription. These observations contrast previous reports of low levels of mtDNA methylation.\textsuperscript{64,65} Furthermore, gene-specific effects of mtDNA methylation were recently associated with the severity of nonalcoholic fatty liver disease.\textsuperscript{66}

Increasing appreciation for the roles that mitochondria play in metabolic dysfunction, as well as other disorders, contends a role for aberrant mtDNA modification in disease development and progression.\textsuperscript{67} Reduced hepatic mitochondrial MT-ND6 expression driven by increased cytosine methylation was recently associated with the severity of nonalcoholic fatty liver disease.\textsuperscript{68} Epigenetic silencing of MT-ND6 in the livers of nonalcoholic steatohepatitis compared with patients with simple steatosis implicates methyl-dependent regulation in the pathogenesis and progression of human metabolic disease. Renewed focus on mitochondrial dysfunction combined with recent identification of localized epigenetic machinery that are sensitive to metabolic changes is likely to uncover novel epigenetic associations to metabolic disease.

### Cofactors From Energy Metabolism

Acetyl-CoA generated from glucose and fatty acid metabolism feeds into the TCA cycle to contribute to cellular energy supply. When flux through this pathway exceeds the energy requirements of the cell, enzymatic conversion of TCA-derived citrate to acetyl-CoA forms the initial step in fatty acid biogenesis. Importantly, acetyl-CoA is the essential acetyl group donor to lysine acetylation reactions and both pharmacological and genetic interventions that modify cellular acetyl-CoA concentrations directly affect acetylated proteins including histones.\textsuperscript{69} Because histone acetylation is ubiquitously associated with open chromatin and gene expression, acetyl-CoA links intermediary carbon metabolism with chromatin dynamics and transcription. Table 2 describes mammalian acetyltransferases that could be influenced by changes in acetyl-CoA. Studies have started to elucidate acetyl-CoA–dependent mechanisms underlying direct interpretation of energy metabolism to structural chromatin changes, yet precisely how individual enzymes compete for varying pools of acetyl-CoA remains unclear. Furthermore, the complexity of this energy-state sensing system is amplified by the prevalence and functional significance of lysine acetylation on proteins other than histones that also directly participate in metabolic function and gene regulation. For example, both cytosolic and mitochondrial isoforms of acetyl-CoA synthetase are themselves deactivated by acetylation.\textsuperscript{70,71} Similar post-translational acetylation elevates the enzymatic activities of the P/CAF,\textsuperscript{72} p300,\textsuperscript{73} and MYST acetyltransferases,\textsuperscript{74} as well as lowering that of the SUV39H1 methyltransferase.\textsuperscript{75}

In direct contrast, nicotinamide adenine dinucleotide (NAD\textsuperscript{+}) is an essential cofactor for reactions catalyzed by the highly conserved sirtuin HDAC family. Seven mammalian sirtuins are differentially distributed throughout the cell, with SIRT1, SIRT6, and SIRT7 mainly localized for nuclear functions.\textsuperscript{68} The immense interest surrounding sirtuin function in metabolism stems from their close homology to yeast Sir2\textsuperscript{80} and similar proteins associated with longevity in flies\textsuperscript{89} and worms.\textsuperscript{90} Important studies have identified roles for SIRT1 and SIRT6 in the regulation of mammalian life span and several lines of evidence strongly implicate sirtuins in the life-extending effects of calorie restriction; however, the precise molecular mechanisms are unclear (recently reviewed elsewhere\textsuperscript{82}). Elevated cellular concentrations of this glycolytic cycle metabolite induced by fasting or caloric restriction have been shown to stimulate SIRT1 activity.\textsuperscript{80,84} Whereas transcription factors predominate most of the metabolic studies investigating sirtuins in nuclear transcription networks,\textsuperscript{85} the direct chromatin-modulating effects of metabolically induced changes in NAD\textsuperscript{+} bioavailability are less clear. Notably, cyclic fluctuations of SIRT1-mediated deacetylation of lysines 9 and 14 of histone H3 at the promoters of genes are activated by circadian

### Table 1. Mammalian Methyltransferase Enzymes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Site</th>
<th>Methyltransferase</th>
<th>Transcriptional Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>K26</td>
<td>EZH2</td>
<td>Silencing\textsuperscript{40}</td>
</tr>
<tr>
<td>H3</td>
<td>R2</td>
<td>PRMT6</td>
<td>Silencing\textsuperscript{31}</td>
</tr>
<tr>
<td>K4</td>
<td>Set7</td>
<td>MLL (ALL-1)</td>
<td>Activation\textsuperscript{32}</td>
</tr>
<tr>
<td>K9</td>
<td>SUV39H1</td>
<td>Silencing\textsuperscript{33}</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td>PRMT5</td>
<td>Silencing\textsuperscript{34}</td>
<td></td>
</tr>
<tr>
<td>K9</td>
<td>PRMT5</td>
<td>Silencing\textsuperscript{35}</td>
<td></td>
</tr>
<tr>
<td>K17</td>
<td>CARM1</td>
<td>Activation\textsuperscript{41}</td>
<td></td>
</tr>
<tr>
<td>K27</td>
<td>EZH2</td>
<td>Silencing\textsuperscript{42}</td>
<td></td>
</tr>
<tr>
<td>K27</td>
<td>G9a</td>
<td>Silencing\textsuperscript{43}</td>
<td></td>
</tr>
<tr>
<td>K36</td>
<td>SET2</td>
<td>Activation (elongation)\textsuperscript{44}</td>
<td></td>
</tr>
<tr>
<td>K79</td>
<td>Dot1</td>
<td>Silencing\textsuperscript{45}</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>PRMT1</td>
<td>Activation\textsuperscript{46}</td>
<td></td>
</tr>
<tr>
<td>K20</td>
<td>Pr-Set7 (Set8)</td>
<td>Silencing\textsuperscript{47}</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>cytosine</td>
<td>DNMT1</td>
<td>Silencing (maintenance)\textsuperscript{48}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNMT3a</td>
<td>Silencing (de novo)\textsuperscript{49}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNMT3b</td>
<td>Silencing (de novo)\textsuperscript{50}</td>
</tr>
</tbody>
</table>

Lysine (K), arginine (R).
oscillations of NAD⁺. The other key NAD⁺ consuming enzymes, ADP-ribosyltransferases, have also been shown to co- 
valently ADP-ribosylate core histones. Although the precise effects of this PTM remain to be characterized, possible cross 
talk with other histone tail modifications such as acetylation may be important for chromatin adaptations to metabolic sensing.

Continual resynthesis of NAD⁺ through salvage/recycling pathways maintains the functions of cytosolic and nuclear enzymes. Nicotinamide phosphoribosyltransferase expression, in addition to its role in NAD⁺ salvage and epigenetics, microRNA-34a was recently identified as a coactivator of the circadian regulator CLOCK, to control NAMPT expression and rhythmic clock target in mice. Furthermore, SIRT1-mediated NAD⁺ levels and SIRT1 activity in obese mice by targeting and decreasing nicotinamide phosphoribosyltransferase expression, in addition to its role in directly inhibiting SIRT1 expression. These observations have broader implications for our understanding of the communication and temporal coupling of circadian rhythm and metabolic cycles with the modification of the epigenome.

Amino Acids and the Epigenome

In addition to the central role of methionine in methylation reactions, there is evidence to suggest that changes in protein and amino acid metabolism can be converted to stable patterns of gene expression by influencing the concentrations of key metabolites. Therefore, pathways for amino acid catabolism provide a metabolic link to epigenetic regulation. For instance, ketogenic amino acids leucine and lysine are catabolized to acetyl-CoA and could therefore shape histone acetylation patterns. Similar reactions convert isoleucine, threonine, and the aromatic amino acids phenylalanine, tryptophan, and tyrosine to compounds that produce acetyl-CoA. Similarly, α-ketoglutarate production via oxidative deamination of glutamate by glutamate dehydrogenase could influence chromatin demethylation.

Sensing Metabolic Variations

The 5’ adenosine monophosphate-activated protein kinase (AMPK) plays a key role in the eukaryotic energy monitoring of AMP:ATP ratio. Activation of this enzyme initiates a program of metabolic adaptation to preserve cellular energy and allow the cell to switch between different energetic substrates in response to nutritional environmental variations. AMPK activates ATP-consuming catabolic pathways involved in oxidative metabolism and mitochondrial biogenesis while switching off ATP-consuming anabolic pathways such as protein, cholesterol, and fatty acid synthesis. AMPK transcriptionally regulates energy expenditure by 2 distinct mechanisms that have consequences for the epigenome. AMPK directly associates with chromatin and phosphorylates H2B at serine 36 to activate transcriptional pathways that confer a cellular response to various metabolic and environmental stresses including nutritional deprivation. Under low glucose conditions, AMPK colocalizes with the p38 transcription factor to phosphorylate H2B at the transcribed cell survival genes cpt1c and p21. Second, AMPK activation leads to an increase in cellular NAD⁺ levels, possibly by increasing NAMPT expression. Pharmacological or physiological activation of AMPK increased NAD⁺ levels to coordinately enhance SIRT1 activity, leading to the deacetylation and altered transactivity of downstream SIRT1 targets: peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and forkhead transcription factors FOXO1 and FOXO3a. These changes in transcription factor deacetylation and consequent activity elevate the expression of mitochondrial and lipid oxidation genes in low glucose and may represent a mechanism by which gene programs are poised for response to energy demands. Furthermore, pharmacological activation of AMPK significantly reduced the levels of superoxide induced by...
palmitate in human endothelial cells.\textsuperscript{112} Although the effects of AMPK-mediated elevation of NAD$^+$ are yet to be demonstrated at the level of post-translational chromatin modification, this axis may represent an important pathway controlling deacetylation and ADP-ribosylation of histone proteins, with potential epigenome-wide effects. A recent study describes downregulation of SIRT1/AMPK commensurate with global enrichment of acetylated H3 histones in the aortas and hearts of rats with postmenopausal metabolic syndrome.\textsuperscript{113}

Another recent connection between energy state and the epigenome operates through the nutrient-responsive hexosamine biosynthetic pathway, which is acutely sensitive to cellular levels of ATP, carbohydrate, lipid, and amino acids. The major end product of this pathway, uridine diphosphate N-acetylgalactosamine, is the precursor to amino sugars used in the synthesis of glycoproteins and glycolipids. Many nucleocytoplasmic proteins are reversibly modified at serine and threonine residues by $O$-linked addition of $N$-GlcNAc ($O$-GlcNAcylation) mediated by a pair of highly conserved enzymes: the $O$-GlcNAc transferase and the $O$-GlcNAcase.\textsuperscript{114} $O$-GlcNAc confers a variety of effects on substrate proteins, and regulated cycling of this PTM is uniquely positioned to transmit the global nutritional status of the cell. $O$-GlcNAcylation of TETs was recently demonstrated to promote TET3 and inhibit enzymatic activity,\textsuperscript{115} potentially linking the hexosamine biosynthetic pathway with DNA-methyl patterns.

$O$-GlcNAc has long been associated with gene regulation and chromatin structure including transcription factors and RNA polymerase II.\textsuperscript{116,117} A classical epigenetic role for this modification has recently emerged from the identification of distinct sites on H2A, H2B, H3, and H4 histones that are differentially marked by $O$-GlcNAc through mitosis.\textsuperscript{118,119} $O$-GlcNAcylation of threonine-32 competitively regulates mitosis-specific phosphorylation of the same residue, as well as serines-28 and -10 of H3 histones to control G2-M cell cycle transition.\textsuperscript{119} Unexpectedly, TETs were shown to facilitate $O$-GlcNAc transferase chromatin localization and global $O$-GlcNAcylation,\textsuperscript{120} as well as influence H3K4me3 through $O$-GlcNAc transferase and SET1/COMPASS at transcription start sites and CpG-rich regions.\textsuperscript{121} Further linking the hexosamine biosynthetic pathway with chromatin architecture, $O$-GlcNAc enhances the stability and histone methyltransferases activity of EZH2.\textsuperscript{122} Newly developed quantification techniques allowing systems level profiling of $O$-GlcNAcylation will considerably enhance our understanding of the metabolic link to gene regulation.\textsuperscript{123} Interestingly, $O$-GlcNAcase also possesses intrinsic HAT activity\textsuperscript{62,88} and could, therefore, regulate acetylation in addition to histone $O$-GlcNAcylation.

\textbf{Chromatin Modifications Associated With Glucose Metabolism}

Endocrine- and receptor-mediated signaling cascades tightly regulate glucose homeostasis to maximize the available energy from carbohydrate intake and glycogen stores. Elevated blood glucose signals epigenetic activation of genes implicated in glucose uptake to peripheral tissue, and disruption by genetic or acquired mechanisms confers numerous vascular pathologies associated with a substantial epigenetic component.\textsuperscript{124} The role of insulin in stimulating glucose uptake is a basic tenet of carbohydrate metabolism that initiates in the pancreatic $\beta$-cell where glucose activates transcription from the \textit{insulin (Ins)} gene. In accordance with the acute response of this gene to environmental signals, the \textit{Ins} promoter is subject to numerous regulatory epigenetic modifications. In addition, specific corecruitment of epigenetic modifiers by islet-enriched sequence-specific factors drives structural chromatin changes. The Pdx1 transcription factor, which plays well-defined roles in pancreas development and $\beta$-cell maturation,\textsuperscript{125} influences the chromatin landscape to activate \textit{Ins} expression by recruiting the p300 HAT to acetylate H4 histones in response to glucose.\textsuperscript{126,127} Similar Pdx1-mediated corecruitment of the Set7 lysine methyltransferase to the \textit{Ins} and \textit{Glut2} promoters is necessary for maintenance of H3K4 methylation and RNA polymerase II elongation required for transcriptional activation.\textsuperscript{128,129} Reciprocally, hypoglycemic conditions confer retention of a proportion of Pdx1 at the \textit{Ins} promoter where it recruits HDAC1 and HDAC2 to deacetylate H4 histones and repress \textit{insulin} expression.\textsuperscript{130} Insulin secretion and signaling activate adipose and muscle cell glucose transporters that facilitate glucose uptake. During adipogenesis, DNA methylation of the glucose transporter 4 (\textit{Glut4}) gene is reduced to enhance chromatin accessibility.\textsuperscript{131} The importance of the DNA-methyl mark at this gene was further demonstrated by reduced expression of \textit{Glut4} in cells lacking estrogen receptor $\beta$ mediated by hypermethylation of a single CpG dinucleotide at an SP1 binding site on the \textit{Glut4} promoter.\textsuperscript{132}

\textbf{Some Memories Are Harder to Forget}

Disruption of this principal system of glucose sensing and uptake has severe long-term health consequences. Chronic hyperglycemia leads to a host of diabetes mellitus–specific pathologies primarily affecting inflammatory and mitochondrial superoxide pathways in the microvasculature of the retina and renal glomeruli, as well as accelerated atherosclerosis and cardiovascular disease.\textsuperscript{133,134} Large-scale clinical trials examining strict and conventional regimens of blood glucose control in type 1\textsuperscript{135,136} and type 2\textsuperscript{137} diabetes mellitus show the decisive benefits of early and stringent intervention. Moreover, these studies define a memory of previous periods of hyperglycemia, such that vascular complications continue to develop despite more than a decade of blood glucose normalization.\textsuperscript{11,12} Studies of experimental animal models and cultured human cells further highlight the persistence of transient and sustained hyperglycemia.\textsuperscript{124} For their ability to stably integrate metabolic information with gene expression, epigenetic chromatin adaptations are increasingly appreciated in this phenomenon of metabolic memory.

Various cell types implicated in vascular dysfunction exhibit distinct patterns of histone methylation likely associated with individual gene expression profiles and potentially modulated by glucose exposure. Hyperglycemic conditions mediate chromatin changes underlying altered gene transcription in vascular and inflammatory cells.\textsuperscript{137,138} Building on clinical and experimental findings, studies of glycemic variability revealed important epigenetic changes driving continued activation of the NFkB-p65 subunit (\textit{REL}) gene and downstream inflammatory promoters. H3K4 methylation by Set7 was shown to
sustain RELA expression induced by transient high glucose in cultured endothelial cells.\textsuperscript{139,140} Numerous glucose sensitive histone-dependent and -independent gene changes were attributed to Set7 in the vascular endothelial cell,\textsuperscript{138} including \textit{HMOX-1} which was recently identified as a predictor of human metabolic disease.\textsuperscript{141} To this end, we recently described widespread pathway deregulation in Set7-depleted human endothelial cells associated with both histone changes and methyl-dependent modulation of transcription factors\textsuperscript{8} that may be important for diabetes mellitus complications.\textsuperscript{27} Persistent H4 methylation at proximal and distal regulatory elements was shown to sustain manganese superoxide dismutase (SOD2) repression in retinal endothelial cells.\textsuperscript{142} Similarly, high glucose-mediated upregulation of \textit{IL-6} in cardiomyocytes was attributed to decreased levels of H3-lysine-9 trimethylation and SUV39H1 expression that persisted in normal glucose.\textsuperscript{143} More recently, genome-wide increases in monocyte H3 acetylation were associated with conventional treatment compared with intensive treatment group subjects of the Diabetes Control and Complications Trial (DCCT), indicating a possible mechanism of metabolic memory in humans.\textsuperscript{144} As most chromatin modifications can be dynamically written and erased, distinguishing persistent and transient epigenetic responses to metabolic change is a key challenge. At this stage, it is unclear precisely how the epigenetic machinery senses glycemic variability. Are transient spikes in glucose concentration sufficient to confer a legacy in human diabetes mellitus? Patients with diabetes mellitus can experience severe fluctuations in blood glucose, and the level of hyperglycemic exposure required to establish future cell memories has not been defined. However, what broader effects do periods of hypoglycemia have on epigenetic gene regulation?

For over a decade, studies exploring the role of metabolism and disease have been limited to cell type–specific epigenetic modifications. After the identification of distinct transcriptional events associated with distinguishable chromatin modifications, the challenge now is to understand their cell-specific function. Whereas histone modifications can be informative of gene regulation at specific loci, this hardly exhausts the broad regulatory potential. To illustrate this, we provide a specific example of genome-wide activating (H3K4me3) and inactivating (H3K27me3) histone marks of human vascular endothelial cells and monocytes (Figure 3). Genome-wide maps generated from soluble chromatin immunoprecipitation combined with sequencing reveal important histone methylation patterns in different cell types. Although there are many sites of trimethylation overlap, not surprisingly we observe unique sites that distinguish vascular endothelial cells and monocytes further highlighting the importance of the epigenome and ongoing ENCODE (The Encyclopedia of DNA Elements) project. A comprehensive review of the datasets shows that there are 22,347 sites of H3K4me3 that overlap both cell types, whereas 18,601 sites are unique to endothelial cells and 42,184 sites specific to monocytes. H3K4me3 marks cover \(\approx 4.8\%\) of the mappable genome in vascular endothelial cells and 6.9% in monocytes. In contrast, there is 16,660 sites of H3K27me3 that overlap both cell types, whereas 36,944 are unique to endothelial cells and 109,162 specific to monocytes. Despite these large differences, H3K27me3 marks cover \(\approx 27.8\%\) of the mappable genome in endothelial cells and 19.1% in monocytes. Although there are fewer H3K27me3 sites in endothelial cells, these regions are longer in length than in monocyte cells. Broad H3K27me3 peaks across the gene body are repressive, whereas short peaks at the transcription start site are associated with bivalent genes.\textsuperscript{145} Endothelial cells may have more broad peaks because they are a terminally differentiated cell type, whereas monocytes may have more short peaks and poised for macrophage differentiation. These plots highlight the cell-specific nature of the epigenome.

But what are the gene targets for active and inactivating marks in different cell types? Their significance are appreciated for the insulin-like growth factors (IGFs), which are considered to be intricately controlled and relevant to the growth hormone axis and regulation of metabolism. The expression of the IGF1 and IGF2 receptors, as well as family members of the IGF2 mRNA-binding proteins (IGF2BP) 1 to 7, show specific H3K4me3 enrichment at their promoters in monocytes and vascular endothelial cells. Whereas the IGF2 hormone is secreted in the liver and known to circulate in blood, these histone marks are tissue specific. The IGF2 gene promoter is enriched for H3K4me3 in human vascular endothelial cells but not in monocytes. Interestingly, H3K4me3 is also enriched at the promoter of IGFBP3, which has been shown to interact with IGF2. Comprehensive genome-wide studies will illuminate the roles of the functional elements and distinguish epigenetic changes in the human genome that explain tissue-specific gene regulation.

Dysregulated \(O\text{-GlcNAc}\) cycling is also associated with the metabolic abnormalities of diabetes mellitus including insulin resistance,\textsuperscript{146} exemplified by the association of risk for type 2 diabetes mellitus with a polymorphism in the \(O\text{-GlcNAc}\) case gene.\textsuperscript{147} Hexosamine biosynthetic pathway overactivity is proposed as a major contributor to diabetic vascular pathology,\textsuperscript{148} and \(O\text{-GlcNAc}\) is accordingly associated with many proteins implicated in diabetes mellitus complications.\textsuperscript{149} Furthermore, hyperglycemic conditions were shown to mediate excessive \(O\text{-GlcNAc}\) ylation of mitochondrial proteins leading to impaired mitochondrial function in cardiomyocytes.\textsuperscript{150} Future studies will determine the importance of histone \(O\text{-GlcNAc}\) ylation to vascular complications.

Epigenetics of Lipid Metabolism

Recent decades have witnessed an astonishing rise in the prevalence of obesity and metabolic dysfunction paralleled by increased consumption of high-energy, fat enriched diets. Fatty acids are important sources of energy particularly during periods of elevated metabolic demand. However, high serum lipid levels are associated with predisposition to an inflammatory state and other metabolic complications including liver steatosis.\textsuperscript{151} Dietary fat intake has been extensively explored in experimental models of metabolic function and disease, revealing key pathological changes in gene expression. More recently, there has been an explosion of studies aiming to characterize the epigenetic components of lipid metabolism in adults as well as the developing fetus.\textsuperscript{21}

As the main site of \(\beta\)-oxidation and fatty acid synthesis in the body, understanding dietary effects on hepatic epigenetic regulation is a key focus of animal studies examining high-fat diet (HFD). Recent data links HFD with widespread chromatin
remodeling at regulatory regions of concomitantly altered genes in mouse livers. Animals fed HFD for 8 weeks exhibited differential expression of >300 genes significantly associated with regions of nucleosome depletion. Lipid metabolism networks were strongly enriched for deregulated gene expression, and the greatest chromatin changes were associated with hepatic transcription factors Hnf4α, Foxa1, and C/ebpα. Livers of rats that developed obesity on HFD exhibited altered histone
modification patterns at promoters and coding regions of cellular senescence genes p16 and p21. 153 Precisely, how these modifications are regulated remains to be determined 152; however, there is evidence to suggest that HFD could initiate epigenetic changes by regulating genes that encode histone modifying enzymes. High fat-fed transgenic adult mice expressing the human cholesterol transporter APOE2 (hAPOE2) gene exhibited significantly elevated hepatic lipid accumulation.154 Extensive global transcriptome changes accompanying this phenotype included elevated expression of histone deacetylase Hdac6, as well as histone demethylases Kdm3b, Kdm5b, and Kdm5c. The importance of specific HDAC activity for hepatic liver homeostasis was previously demonstrated by studies of knockout animals.155 Similarly, gluconeogenesis and ketogenesis pathways are associated with regulators of histone methylation.156,157

An important consideration is not only the total fat content of the diet, which varies greatly across studies,158 but also the influences of specific fatty acids on gene expression. Although several studies describe the effects of well-defined, semipurified HFDs, there are no reports that definitively distinguish the epigenetic effects of specific dietary fatty acids in vivo. However, a small number of recent investigations are starting to link chromatin changes with lipid-responsive genes in cultured cells of various origins. Paralleling the hAPOE2 mouse, cultured primary mouse hepatocytes loaded with palmitate and oleate expressed histone demethylase genes at elevated levels.159 Chromatin immunoprecipitation linked these expression changes with disrupted patterns of H3-lysine-9 trimethylation and H3K4me3 modifications at hepatic lipid catabolism network genes. Similarly, docosahexaenoic acid decreased expression of HDAC1, HDAC2, and HDAC3 and induced global histone changes associated with transcriptional activation including H3-lysine-9 acetylation enrichment as well as depletion of H3-lysine-9 and -27 dimethylation in human neuronal cells.159 Interestingly, docosahexaenoic acid was also shown to increase expression of H3 and H4 histones in the same cell line.160 By contrast, palmitate induced HAT activity, and several genes that were modulated by lipotoxicity displayed altered patterns of histone modification in clonal β-cells.161

Whereas genome-wide influences of specific fatty acids are largely unknown, extensive effects of palmitate on DNA-methyl-dependent transcription were recently demonstrated for human pancreatic islets. Significant changes in DNA methylation were associated with altered expression of 290 genes after incubation for 48 hours with palmitate.162 As well as differential regulation of numerous metabolic pathways including insulin signaling, several genes associated with type 2 diabetes mellitus exhibited DNA methyl-dependent expression changes. Incubation of primary human skeletal muscle cells with palmitate and oleate induced DNA hypermethylation and repression of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) gene promoter.163 Silencing of DNMT3B prevented the response to palmitate at the promoter of this regulator of mitochondrial biogenesis and attenuated the repression of other palmitate-responsive genes important for mitochondria function.

Endogenous HDAC Inhibition
Further investigations are clearly required to determine precisely how long-chain fatty acids mediate chromatin changes. By contrast, the histone deacetylation inhibitory properties of the short chain fatty acid sodium-butyrate have long been known.164 Derived from anaerobic microbial fermentation of dietary fibers in the colonic lumen, butyrate provides an important energy source for intestinal epithelial cells and regulates numerous cellular functions165 including protection against inflammatory disease166 and colorectal carcinogenesis.167 HDAC inhibition and subsequent hyperacetylation of genes such as p21 is thought to underlie the apoptotic and antiproliferative effects of butyrate in cancer cells.168 However, the precise mechanism of action is unclear, and in addition to direct interaction with HDACs, butyrate is proposed to act as a product inhibitor of deacetylation.169 Recent studies indicate that butyrate can also stimulate HAT activity in colorectal cancer cells where it is metabolized to acetyl-CoA.170 Moreover, by manipulating oxidative metabolism, Donohoe et al170 demonstrated that the metabolic state of the cell determines the balance of HDAC inhibitory and HAT stimulatory effects of butyrate. More recently, an endogenous metabolic product of fatty acid oxidation bearing structural homology to butyrate has emerged as an important endogenous HDAC inhibitor.171

In addition to providing substrate for the TCA cycle, fatty acids are converted to ketone bodies for export as a glucose-sparing energy source to metabolically active tissues. Under normal conditions, ketones are produced at relatively low rates in well-nourished individuals. However, during extended caloric restriction, starvation, or intense exercise,172 depletion of TCA cycle intermediates by gluconeogenesis coupled with increased mobilization of fatty acids to the liver divert acetyl-CoA to ketone production. This allows continued β-oxidation and energy transport to extrahepatic tissue when acetyl-CoA accumulates beyond the capacity of the TCA cycle. Serum ketone concentrations rise from μmol/L basal levels to 1 to 2 mmol/L after intense exercise,173 fasting,174 or in response to a low carbohydrate diet.175 The most abundant circulating ketone, β-hydroxybutyrate (βOHB), is regarded as the predominant source of energy during prolonged exercise or calorie restriction.176 βOHB differs structurally from butyrate by a single hydroxyl group, and in addition to its intermediary role in energy metabolism, has recently emerged as an endogenous and specific inhibitor of histone deacetylation.177 Human embryonic kidney cells exhibited dose-dependent enrichment for H3 histones acetylated at lysines-9 and -14 (H3-lysine-14 acetylation) after 8-hour stimulation with βOHB at physiologically relevant concentrations. Furthermore, βOHB dose dependently inhibited deacetylase activities of recombinant human HDAC1, HDAC3, and HDAC4 in vitro.

Calorie-restricted mice displayed significant enrichment for H3K9ac particularly in the kidney, in strong correlation with serum βOHB concentration.178 High fat, low calorie ketogenic diets are broadly considered to enhance neuron resistance to diverse cellular injury including damage induced by oxidative stress.179 Accordingly, transcriptome profiling revealed overlap of numerous renal genes increased in response to either βOHB or fasting, including several genes implicated in oxidative stress resistance.177 Chromatin immunoprecipitation experiments revealed that βOHB-mediated activation of oxidative stress resistance genes Foxo3 and MT2 genes was driven by specific inhibition of HDAC1 and H3K9ac enrichment.
To this end, continuous delivery of βOHB for 24 hours protected murine kidneys against oxidative stress–induced amino acid carboxylation and lipid peroxidation. The extensive epigenome editing effects of ketogenic diets are further exemplified by dietary modulation of DNA methylation to attenuate seizure progression in a rodent model of epilepsy.  

### Pharmacological Deacetylation

Anyone researching chromatin modification, specifically histone acetylation, knows about the impact of pharmacological HDAC inhibition, suppressing enzymatic activity impinging on the chromatin template serving to increase total histone acetylation and activate gene expression. After decades of studying acetylation dependent gene expression, often at single loci, the challenge faced by scientists was determining the impact of histone acetylation as the paradigmatic mechanism of action. With the advent of next generation sequencing, genome-wide histone maps and transcriptomic analyses have exposed some unexpected findings, stimulating new areas of research in gene regulation. Pharmacological HDAC inhibition in mammalian cells shows a complex pattern of gene expression changes that were associated with histone acetylation and deacetylation events (Figure 4). Genome-wide maps show surprising deacetylation by the hydroxamic acids, trichostatin A and suberoylanilide hydroxamic acid (SAHA, also known as Vorinostat and Zolinza). SAHA was the first pharmacological HDAC inhibitor granted regular approval by the US Food and Drug Administration for the treatment of cutaneous manifestations of T-cell lymphoma. The identification of broad H3K9/14 deacetylation by trichostatin A and SAHA in primary human aortic cells and the use of the EP300/CREBBP-specific inhibitor provided further mechanistic clues. The inhibition of EP300/CREBBP activity was associated with transcriptional events regulating gene expression. The direct loss of EP300/CREBBP at target genes was consistent with histone deacetylation by trichostatin A. These, and future descriptions of the regulatory pathways critical to transcription, should help provide a framework to further understand the complex signaling mechanisms relevant to vascular endothelial function for therapeutic purposes.

### Maternal Diet Shapes More Than Waistlines

Seminal observations associating birth weight with cardiovascular disease risk led researchers to hypothesize a relationship between in utero environmental programming and long-term health outcomes. According to the Developmental Origins of Health and Disease model, adaptive responses to intrauterine conditions in anticipation of postnatal environments confer increased risk of chronic disease in adulthood. Both undernutrition and overnutrition throughout gestation are associated with increased susceptibility to adult onset of metabolic dysfunction including diabetes mellitus and metabolic syndrome, emphasizing the importance of dietary balance during development. At the level of public health, the alarming escalation of obesity and metabolic disease underscores the need to characterize the influence of a mother’s diet on the long-term health of her children. Because chromatin marks established during prenatal and early postnatal development can respond to local changes in nutrient availability and metabolite concentration, epigenetic variation introduced by gestational cues have strong capacity to define and direct persistent metabolic expression profiles.

### Undernutrition In Utero

Disastrous periods of famine provide historical and biological insight into gestationally programmed disease. Most striking were the results of a recent total population study that revealed massively increased risk for diabetes mellitus in those born during and immediately after 3 major famines in Austria over the last century. The timespan between intrauterine development and adult disease onset limits the direct evidence of epigenetic mechanisms underlying maternal nutrient programming of metabolic function in humans. Furthermore, the relative contributions of genetic and environmental factors to the developing human epigenome are difficult to distinguish in outbred populations. The vast majority of findings linking transient environmental conditions in gestation to epigenetic changes in adult disease are therefore derived from experimental animal models of dietary modulation. A prominent exception concerns a cohort of individuals conceived during the Dutch famine in the winter of 1944 to 1945. Although genome-wide analysis did not support a relationship between global DNA methylation and prenatal famine exposure in white blood cells of this cohort, a separate study found that periconceptional starvation was associated with decreased CpG methylation at the maternally imprinted IGF2 gene in whole blood compared with unexposed, same-sex siblings. By contrast, exposure to famine late in gestation was not associated with methyl changes at this locus, highlighting the importance of early development to epigenetic programming. Such gene-specific changes measured 60 years after the in utero metabolic insult emphasize the capacity for epigenetic modifications, and particularly the robust DNA-methyl mark, to predict adult health outcomes from early life exposures. Similar findings at the Igf2 promoter in rodent hepatocytes were described in models of growth restriction during intrauterine development. Contrasting classical DNA-methyl-dependent silencing, hypermethylation of a distal CTCF binding site at an imprinting control region eliminates the insulating effect of CTCF to allow access of the Igf2 promoter to downstream enhancers and transcriptional activation. Maternal low-protein diet induced hypermethylation at the imprinting control region in strong association with increased Igf2 gene expression at birth. Further parallelling human studies, global DNA methylation patterns including the CpG-rich differential methylation region 2 of Igf2 were unaltered by maternal diet. Intriguingly, expression of DNA methyltransferases Dnmt1 and Dnmt3a as well as the methyl-CpG binding protein Mbd2 were elevated by low-protein diet in utero. Regulation of liver Igf1 in models of intrauterine growth restriction was similarly associated with DNA hypermethylation. Specifically, utero-placental insufficiency affected sex-specific genomic methylation and histone modification patterns along the Igf1 gene that persisted postnatally and concurrent with decreased hepatic Igf-1 mRNA and serum protein levels. Interestingly, differential DNA methylation was also recently implicated in deregulated Igf gene expression in offspring born to mothers with impaired glucose tolerance. Other examples of intrauterine growth retardation
and methylation-dependent silencing in rats include genes that code for the glucocorticoid receptor in both the hippocampus and liver, as well as pancreatic Pdx1. More recently, Pgc-1α was shown to be silenced by DNA hypermethylation, and Glut4 expression was altered by derangement of histone acetylation and methylation patterns in skeletal muscle of female pups exposed to low protein in utero.

Overnutrition and the Developing Epigenome

Studies linking overnutrition with epigenetic changes have also recently extended to models of maternal diet and gestational programming. Dietary essential fatty acids must first be metabolized for synthesis of larger fatty acids in the liver, encoded by FADS1 and FADS2, respectively. Furthermore, synthesis of polyunsaturated fatty acids by Δ-6 desaturase in the vascular endothelium was recently associated with vasoconstriction in rats. DNA hypermethylation was previously found to silence rodent liver Fads2 expression and consequently decrease docosahexaenoic acid and arachidonic acid synthesis by reduced capacity to desaturate precursor fatty acids. Similar epigenetic regulation at a putative intergenic enhancer was recently described for the adjacent FADS genes in human liver biopsies. Reduced expression of this enzyme in aortas of adult offspring born to rats fed HFD throughout pregnancy and lactation was strongly associated with hypermethylation of a single CpG dinucleotide located at position -394 of the Fads2 transcription start site. Compared with soybean oil, long-term dietary supplementation of fish oil similarly repressed Fads2 expression in female rats by reversible methylation of this region. Importantly, Fads2 silencing conferred by methylation of 4 distinct CpG dinucleotides was also observed in male and female offspring, with the strongest association at the -394 CpG site.

Recent observations indicate that maternal exercise before and during gestation could alter HFD-induced methyl-dependent gene changes in offspring. Paralleling HFD in adults as well as maternal protein restriction, transcriptional repression of Pgc-1α in mice born to dams-fed HFD persisted into adulthood, concomitant with sustained methylation of a CpG site within the promoter. Remarkably, voluntary exercise for 6 weeks before and during pregnancy significantly attenuated these epigenetic changes to improve Pgc-1α expression in the skeletal muscle of offspring, but not livers.

Figure 4. Pharmacological histone deacetylase (HDAC) inhibition confers gene expression by acetylation and deacetylation of H3K9/14. Cells stimulated with the pan-HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) and the class I specific HDAC inhibitor romidepsin regulate broad gene expression changes. Shown here are genome-wide H3K9/14ac profiles derived by chromatin immunoprecipitation (ChIP)-seq from human aortic endothelial cells (HAEC) stimulated with SAHA and human colon cancer cells (HCT116) exposed to SAHA and romidepsin. Active gene expression correlated with H3K9/14 acetylation are shown in blue, whereas inactive genes correlated with H3K9/14 deacetylation are shown in orange. High throughput gene expression and ChIP-seq data are publically available and accessed from Gene Expression Omnibus (ID: GSE22061 and GSE37378).
Figure 5. Mapping cell-specific histone acetylation. This plot of human chromosome 12 shows the variation of histone H3 lysine 9 acetylation (H3K9ac) among cell types. The outer ring represents ~134 million base pairs showing some of the estimated 1200 protein coding genes represented in blue and red, including long intergenic nonprotein coding RNA (lncRNA) and short noncoding or micro RNA (miRNA). Differential H3K9ac is represented in each concentric ring shown in blue bar charts for endothelial (HUVEC, human umbilical vein endothelial cells), monocytes (CD14+), myoblasts, keratinocytes, astrocytes and stem cells. ChIP-seq profiles for H3K9ac are derived from ENCODE and shown in blue. The innermost track represents aortic endothelial cells showing histone acetylation (green) and deacetylation (orange) conferred by pharmacological HDAC inhibition. Names of genes, lncRNAs and miRNAs associated with differential H3K9 acetylation and deacetylation at the promoter are shown. Histone acetylation data are generated by chromatin immunoprecipitation combined with sequencing (ChiP-seq) derived from ENCODE and accessed from UCSC. Genes implicated with metabolism and vascular function are highlighted in red and cross-referenced with function in Table 3.
Further investigation of this maternal condition on metabolic dysfunction in offspring could reveal important information for human metabolic health. By contrast, the deleterious effects of maternal HFD on glucose intolerance and insulin resistance were exacerbated by folic acid supplementation, concurrent with increased global DNA methylation in mouse adipose tissue. The effects of maternal folate diet are complicated and require further study, with both low and high folate intake associated with increased disease risk in adult offspring. For instance, standard recommended maternal daily intake of 0.4 mg/d was associated with lower birth weight and altered DNA methylation of IGF2.

Finally, the importance of histone acetylation for gestational programming by HFD is also highlighted by several recent studies. Hdac3 recruitment was associated with H4 deacetylation and p16 repression in mammary glands from female offspring of rats fed an HFD throughout pregnancy and during lactation. By contrast, persistent Hdac1 repression and gene-specific H3K14 hyperacetylation, as well as changes in Sirt1 expression, were observed in the fatty livers of Japanese macaques exposed to maternal HFD. In accordance with differential expression of Sirt1, Gen5, Hdac1, and Hdac3, enrichment of histone acetylation, and also histone methylation, was observed at promoters of genes important for lipid metabolism in livers of fetal and 5-week offspring. Similar histone lysine modifications were associated with developmentally programmed changes in leptin and adiponectin expression in adipose tissue.

Current Perspectives on Metabolic Pathways

The immense interest in metabolism and chromatin-dependent gene changes will undoubtedly uncover many additional connections to epigenetic regulation. Despite recent advances in our understanding of glucose and lipid metabolism, we are only beginning to scratch the surface of this evidently complex system and several technical challenges delay a comprehensive understanding.

Although metabolite intermediates provide numerous mechanistic associations to chromatin regulation, the importance of subcellular compartmentalization of epigenetic cofactors should not be overlooked. For instance, fluctuating concentrations of TCA cycle intermediates may directly influence mitochondrial protein PTMs; however, it is the cytoplasmic/nuclear levels of these molecules that are likely to affect epigenetic signaling. As well as specific mechanisms of mitochondria-cytosol transport that couple cofactor synthesis and chromatin modification may strengthen the epigenetic association to metabolism. The broad substrate specificity of most histone modifying enzymes confers an ability to regulate gene expression profiles by chromatin-dependent mechanisms as well as PTM of nonhistone proteins such as nuclear receptors, transcription factors, and importantly histone modifying enzymes. The dynamic and extensive relationships between DNA methylation, histone PTMs, noncoding RNA, and transcription factor networks emphasize the need for a systems perspective of metabolism. And although important enzymes and chromatin changes have been described, defining the specific process governing the cell’s ability to sense environmental change in development and adulthood at the level of gene expression is an important challenge facing the field. Precisely, how are environmental changes signaled to the operational epigenetic machinery? The prevalence of PTMs occurring on HATs, HDACs, histone methyltransferases, and demethylating enzymes suggests a highly ordered interactive network comprising many components capable of adding and removing modifications at both the chromatin template as well as each other. The sensitivity to metabolite fluctuation of interenzymatic modification preceding the instruction of chromatin changes raises the important question, Who regulates the regulators? Paralleling the histone code, mapping the extrachromatin PTM cipher controlling organ(elle)-specific and metabolically contextualized enzyme function, stability, and consequent chromatin-modifying activity holds immense potential to further our understanding of environment–gene interaction.

Reminiscent of early single-loci genetic studies, many epigenomic investigations have examined specific modifications in isolation. Increased availability of public epigenome-wide datasets has greatly enhanced our understanding of the chromatin landscape. Nonetheless, there are clear advantages of simultaneous profiling of multiple chromatin modifications in conjunction with gene expression for the generation of detailed cell-type and stimuli-specific epigenomic maps (Figure 5). The data represented in Table 3 highlight not only the remarkable parallels across distinct cell types but also the prominent differences between tissues, and the enrichment for genes associated with metabolism and vascular function. The relevance of epigenetic variation among tissue types becomes apparent in large well-phenotyped sample cohorts.

Future Considerations for the Clinic

Determining the genetic and nongenetic origin of complex human disease remains a fundamental challenge to biomedical research. Whereas genome-wide association studies have uncovered loci with SNP (single nucleotide polymorphism) associations, it is becoming increasingly appreciated that nongenetic variability or gene–environment interactions could influence complex disease pathogenesis, which is now the subject of intense debate. Using powerful profiling techniques, there exists opportunities for scientists to explore epigenome-wide association studies for human diseases. Setting aside the technological bottleneck of the past, the techniques developed for genome-wide profiling of chromatin modifications as well as genomic methylation has seen tremendous advances. The last decade has seen the availability of profiling technologies for genomic methylation and with the advent of high throughput sequencing and improved array-based techniques has meant greater choice when balancing design tradeoffs such as genome coverage and resolution as well as specificity and experimental costs. And while there are options for epigenome-wide profiling that use array-based technologies for signal detection, there are many more choices for methyl-capture or direct methylation-sequencing approaches. Hence, there is a real need for a systematic approach to study the human methylome based on profiling technologies and a consensus in handling large-scale datasets. But is epigenetic change sufficient to explain the origin of metabolic disease? Although unreasonable at
this stage to discount the epigenome, it also remains ambiguous to pinpoint epigenetic variation in disease without beginning to understand its significance in human health.236,237 And although large population methylome studies that involve scientists, clinicians, and large consortiums are well underway, the results of a recent epigenetic-epidemiology study provides some insights to DNA methylation and body mass index (BMI).238

Table 3. Genes Identified in Metabolism and Vascular Function and Disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Related Function and Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTF3</td>
<td>Diabetic polyneuropathy239</td>
</tr>
<tr>
<td>SLC2A3</td>
<td>Gestational diabetes210</td>
</tr>
<tr>
<td>CREBL2</td>
<td>Glucose homeostasis, adipose cell differentiation by CREB1211</td>
</tr>
<tr>
<td>PDE3A</td>
<td>Cardiovascular function by regulating smooth muscle contraction and relaxation212,213</td>
</tr>
<tr>
<td>ARNTL2</td>
<td>Hypoxia and angiogenesis214,215</td>
</tr>
<tr>
<td>FAR2</td>
<td>Fatty acid metabolism216</td>
</tr>
<tr>
<td>PKP2</td>
<td>Arrhythmogenic right ventricular dysplasia217</td>
</tr>
<tr>
<td>ALG10</td>
<td>Long-QT syndrome218</td>
</tr>
<tr>
<td>SLC2A13</td>
<td>Facilitated glucose transporter219</td>
</tr>
<tr>
<td>RAPGEF3</td>
<td>Angiogenesis and endothelial barrier function220</td>
</tr>
<tr>
<td>PPP1R1A</td>
<td>Glycogen metabolism and developmental insulin resistance221,222</td>
</tr>
<tr>
<td>GPR1B2</td>
<td>Binds potent adrenomedullin vasodilator peptide exerts cardiovascular function223</td>
</tr>
<tr>
<td>HMG2A</td>
<td>Transcriptional regulator essential for normal cardiac development224</td>
</tr>
<tr>
<td>TSPAN8</td>
<td>Related to body weight regulation225</td>
</tr>
<tr>
<td>BBS10</td>
<td>Adipogenesis and obesity226</td>
</tr>
<tr>
<td>E2F7</td>
<td>Angiogenesis and VEGFA gene activation227</td>
</tr>
<tr>
<td>GALNT4</td>
<td>Implicated in coronary artery disease (GWAS)228</td>
</tr>
<tr>
<td>KCTD10</td>
<td>Regulates heart morphogenesis by repressing T box transcription factor Tbx5a229</td>
</tr>
<tr>
<td>ATP2A2</td>
<td>Cardiac muscle contraction/relaxation and implicated in cardiac hypertrophy230</td>
</tr>
<tr>
<td>TBX3</td>
<td>Cardiac conduction development231</td>
</tr>
</tbody>
</table>

GWAS indicates genome-wide association studies; and VEGFA, vascular endothelial growth factor A.

Epigenome and Public Health

It is increasingly evident that characterization of the epigenetic interface uniting metabolic changes with gene regulation will greatly enhance our understanding of present-day metabolic disease. What we already know is that stable epigenetic memories established by developmental and adult nutritional milieus have strong associations to disease predisposition and progression, respectively. This is exemplified by chromatin modifications conferred by intrauterine nutrition that persists into adulthood in association with insulin resistance and type 2 diabetes mellitus development. Consequential hyperglycemia can then confer further epigenetic perturbations contributing to chronic vascular complications that are perpetuated beyond restoration of normal blood glucose.214
the sex-specific changes mediated by metabolic gestational programming is another prominent challenge facing the field.240,241 In addition, studies indicate that paternal nutrition cannot be discounted, as HFD was recently associated with altered DNA methylation and β-cell dysfunction in female rat offspring.242 More recently, germ cell DNA hypomethylation associated with the transmission of metabolic dysfunction from diet-induced obese paternal mice to 2 subsequent generations.243 Identification of gestationally determined epigenetic changes associated with metabolic disease in humans provides potential mechanisms to explain disease risk in many historical famine cohorts184,244 and is equally relevant to recent and current famines around the globe. The effects of long-term poverty on changes in body shape245 are likely to include nutritional-epigenetic components. Similarly, rising levels of fat consumption and generational exposure of vulnerable populations to the western diet is liable to impact epigenomes throughout development and adulthood.

The extent to which pathological chromatin changes are reversible remains controversial. Our recent studies of pharmacological HDAC inhibition revealing genome-wide non-canonical effects on chromatin highlight the complexities associated with epigenomic editing. Recent studies have, however, demonstrated the therapeutically potential of distinct classes of HDAC inhibitors in cardiovascular disease.246 Likewise, the potential for epigenetic intervention by targeting lysine and arginine histone methyltransferases is increasing.247 With the rise in massive parallel sequencing complemented by the increased reposition of detailed epigenomic datasets, a clearer image of the responsive chromatin landscape will emerge to result in unprecedented scope and opportunity to therapeutically prevent, attenuate, or reverse the epigenetic disruption associated with metabolic disease.

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None.

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