Reviews

This Review is part of a thematic series on Cardiovascular Complications of Diabetes and Obesity, which includes the following articles:
The Promise of Cell-Based Therapies for Diabetic Complications: Challenges and Solutions [Circ Res. 2010;106:854–869]
Activation of Protein Kinase C Isoforms and Its Impact on Diabetic Complications [Circ Res. 2010;106:1319–1331]
Aldose Reductase and Cardiovascular Diseases, Creating Human-Like Diabetic Complications In An Experimental Model [Circ Res. 2010;106:1449–1458]
Endoplasmic Reticulum Stress and Inflammation in Obesity and Diabetes [Circ Res. 2010;107:579–591]
Oxidative Stress and Diabetic Complications [Circ Res. 2010;107:1058–1070]

Epigenetics: Mechanisms and Implications for Diabetic Complications

Ann Marie Schmidt, Guest Editor

Epigenetics
Mechanisms and Implications for Diabetic Complications

Mark E. Cooper, Assam El-Osta

Abstract: Epigenetic modifications regulate critical functions that underlie chromosome metabolism. Understanding the molecular changes to chromatin structure and the functional relationship with altered signaling pathways is now considered to represent an important conceptual challenge to explain diabetes and the phenomenon of metabolic or hyperglycemic memory. Although it remains unknown as to the specific molecular mechanisms whereby hyperglycemic memory leads to the development of diabetic vascular complications, emerging evidence now indicates that critical gene-activating epigenetic changes may confer future cell memories. Chemical modification of the H3 histone tail of lysine 4 and 9 has recently been identified with gene expression conferred by hyperglycemia. The persistence of these key epigenetic determinants in models of glycemic variability and the development of diabetic complications has been associated with these primary findings. Transient hyperglycemia promotes gene-activating epigenetic changes and signaling events critical in the development and progression of vascular complications. As for the role of specific epigenomic changes, it is postulated that further understanding enzymes involved in writing and erasing chemical changes could transform our understanding of the pathways implicated in diabetic vascular injury providing new therapeutic strategies. (Circ Res. 2010;107:1403-1413.)

Key Words: epigenetics ■ histones ■ chromatin ■ hyperglycemic memory ■ diabetic complications

Diabetic vascular complications remain the major cause of morbidity and mortality in both type 1 and type 2 diabetes. Although much of the original research and indeed the earliest end points in clinical studies of type 1 and type 2 diabetes often focused on microvascular end points such as retinopathy and nephropathy, it is increasingly appreciated not only in type 2 but also in type 1 diabetes that macrovascular disease presenting clinically as myocardial infarction, strokes, and peripheral vascular disease confers a major burden in the diabetic population leading to premature death.¹
There have been a large number of epidemiological studies as well as more recent clinical trials exploring the role of intensive glycemic control in reducing the development and progression of vascular complications in type 1 and type 2 diabetes. Of particular interest were the findings from the Diabetes Control and Complications Trial (DCCT, 1983 to 1993) and its follow up observational study, the Epidemiology of Diabetes Intervention and Complications (EDIC, 1993 to 2006) trial where the term “metabolic memory” was introduced.8-10 This term was used to explain a phenomenon where the long-term vascular benefits of a previous period of good glycemic control persist despite a return to usual, often worse metabolic control. This phenomenon was reported in the DCCT/EDIC trial not only for nephropathy and retinopathy but more recently also with respect to macrovascular disease.11 Contrary to the initial report from the DCCT phase of the trial indicating no differences in cardiovascular events were observed between the intensive and conventionally treated groups, in the EDIC phase, clear differences were demonstrated, with the intensive group having a much lower risk of cardiovascular as well as renal and retinal disease.11

With a cohort of 1441 individuals and a mean follow-up of 6.5 years,12 the DCCT clinical study demonstrated that intensive blood glucose control reduced the onset and progression of long-term diabetic complications, including retinopathy, nephropathy, and neuropathy.13-18 The frequency of cardiovascular events was too low to determine whether the interventions had significantly different effects and therefore the follow-up EDIC study examined the long-term effects of the original DCCT group. The completion of the DCCT study in 1993 transitioned with the launch of the EDIC observational trial in 1994 with some surprising results. During the EDIC study, the difference in glycemic control measured by hemoglobin A1c between the intensive treatment group compared with the conventional treatment group was 2% (7.2% compared 9.1%, respectively) but was now indistinguishable toward the end of the EDIC follow-up study. The study group identified that the benefit of intensive therapy for 6.5 years conducted early had persisted for at least 10 years despite the normalization of hemoglobin A1c, raising the intriguing concept of long-term metabolic memory of previous therapies.9,10,19

Over the last few years, this issue linking glucose levels to diabetic complications has been extensively examined in type 2 diabetes. The phenomenon of metabolic memory has been given the term as the “legacy effect” by investigators from the United Kingdom Prospective Diabetes Study (UKPDS). The authors of the report indicated that the initial modest effect of borderline statistical significance of glucose lowering on macrovascular disease described at the termination of the UKPDS were now much clearer more than 10 years later, despite both the intensive and conventionally treated groups returning to usual glucose lowering management after the completion of the active phase of the trial.20 Thus, based on the findings of the DCCT/EDIC and UKPDS trials, it appears that there is evidence of “metabolic memory” in type 1 and type 2 diabetes, respectively.

More recently, 3 studies, the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation), ACCORD (Action to Control Cardiovascular Risk in Diabetes), and VADT (Veterans Affairs Diabetes Trial), have reported, in general, unremarkable effects of glucose lowering on cardiovascular events, cardiovascular death, and all-cause mortality as a result of glucose-lowering strategies in type 2 diabetes.21-23 However, although the glucose-lowering phase of these trials is completed, the mean follow-up of these trials is much shorter to date than that of the DCCT/EDIC, which identified persistent benefits years later. In view of the potential long-term end-organ effects of metabolic memory, it is predicted that the ongoing follow up of individuals in these trials, as is indeed already in progress for the ADVANCE trial, will confirm whether these trials, which involved periods of at least several years of improved glycemic control, will ultimately confer reduced cardiovascular disease.

The underlying molecular explanation for metabolic memory remains to be fully elucidated. This concept was elegantly demonstrated more than 20 years ago in the seminal studies by Engerman and Kern.24 After 2.5 years of poor glucose control in diabetic dogs, these animals were switched to good glycemic control. However, these animals had the same evidence of retinopathy when compared with dogs subjected to poor glycemic control for the entire period of the study. The study was terminated at 5 years, and therefore any longer-term benefit of intensive treatment might have had on retinopathy progression or regression was not assessed. Although these experiments in diabetic dogs were focused on retinopathy, it is likely that this phenomenon, as reported clinically in the DCCT/EDIC and UKPDS trials, is also seen with respect to nephropathy and atherosclerosis. Indeed, in recent studies by our group in diabetic apolipoprotein E knockout mice, restoration of near normoglycemia after a period of hyperglycemia was associated with persistent ath-
evidence in both metabolic memory and diabetic complications. The importance of MCP-1 and VCAM-1 in diabetic complications is being increasingly recognized, with studies showing that inhibition of these molecules can attenuate inflammatory responses and reduce vascular damage. The ongoing research in this area highlights the potential for developing new therapeutic strategies to prevent and treat diabetic complications.

Role of Oxidative Stress in Mediating Diabetes-Associated Epigenetic Modifications

Oxidative stress is a central player in the development of diabetic complications. High glucose levels are known to increase oxidative stress, which can lead to the formation of advanced glycation end products (AGEs) and reactive oxygen species (ROS). These molecules can activate a range of signaling pathways, including NF-kB, which is a key transcription factor involved in inflammation and immune responses. The role of oxidative stress in mediating epigenetic changes in diabetes is an area of active research, with studies showing that antioxidants can attenuate the deleterious effects of diabetic complications. Further studies are needed to elucidate the complex interplay between oxidative stress, epigenetics, and diabetic complications.
explored using molecular approaches involving the transfection of endothelial cells with adenovirus overexpressing the constructs for manganese superoxide dismutase and uncoupling protein-1. These molecular approaches led to the inhibition of glucose-induced NF-κB activation as a result of reduced p65 gene expression. This attenuation of gene expression appeared to occur as a result of prevention of glucose-induced histone methylation of the p65 gene. To complement this approach, experiments in our laboratory using idebenone, a relatively selective mitochondrial antioxidant, reduced recruitment of the Set7 histone methyltransferase to chromatinized p65 template (Assam El-Osta, unpublished observations, 2008). It remains to be determined whether other sources of ROS, such as cytoplasmic ROS generated by enzymes such as NADPH oxidase, can also influence epigenomic events. This is relevant because NADPH oxidase has been clearly demonstrated to play a role in NF-κB activation in the diabetic context.

Another factor that can influence NF-κB is the key intracellular signaling molecule protein kinase (PK)C. To explore the role of PKC, pilot studies were performed using the nonspecific PKC inhibitor bisindolylmaleimide, which demonstrated reduced H3K4me1 (monomethylation of the histone tail of H3 lysine 4) of the p65 gene sequence with associated abrogation of the glucose induced activation of p65 gene expression (Assam El-Osta, unpublished observations, 2008). With increasing evidence that PKC, specifically the α, β, and δ isoforms, may be relevant to the diabetic context, it will be of interest to further define the role of these enzymes in mediating chromatin remodeling events in the diabetic vasculature.

The Distinguished Genome, Epigenetic Modifications to the Chromatinized Template

The significance of genome structure and function cannot be explained nor understood unless we begin to untwist the DNA strands and examine the core nucleosome components. Indeed, not all genomic sequences are born equally. The genome is in fact characterized by epigenetic sequences that distinguish the polyvalent chromatin fiber, which we and other groups have recently exemplified in specified models of glycemic variability. The posttranslational modification of histone proteins represent chemical variations to the chromatin template, which control sequence structure and gene function. The diversity of modifications as well as the pattern and distribution can reflect different structural and functional roles mediated by epigenetic change.

The multiplicity of modifications represent a complexity of epigenetic regulation, with the best studied being the lysine residue of the histone tail. These amino acids can be distinctly modified by different classes of covalent changes that include acetylation and methylation, phosphorylation, ubiquitination, or sumoylation (Table 1). When, in 1977, the structure of the nucleosome core particle was described, the significance of the original work on nucleosomal crystals heralded a new beginning in chromatin biology and an advancement in our understanding of chromosome structure and function. More than 20 years later, the concept that nucleosomal modifications regulating chromatinized events, now referred to as the “histone-code” hypothesis, was published and has since gained considerable momentum with the realization that nucleosomal histones are distinct in pattern and distinguishable by modification. The acetylation mark was the first posttranslational modification identified on histones, and the impact and significance of research in this area has exploded with the identification of enzymes that can either write or erase these acetylation moieties. The majority of acetyltransferases belong to the GNAT (Gcn5-related N-acetyltransferase) or MYST (MOZ, Ybf2/Sas3, Sas2, and Tip60) families and catalyze the addition of the acetyl moiety from acetyl-coenzyme A to the ε-amino of the lysine residue (Table 2). These covalent modifications are often classically associated with changes in chromatin structure, rendering chromatinized templates more accessible and open, which closely parallel transcriptional competence and gene expression. Indeed the ability to dynamically regulate chromatin is facilitated by the recruitment of critical transcriptional coactivator complexes such as p300/CBP (E1A binding protein p300/CREB binding protein), PCAF (p300/CBP-associated factor), and TAFII250 (transcription initiation factor TFIID 250-kDa subunit), which possess intrinsic histone acetyltransferase activity. The acetylation of histones is also a reversible process and to initiate the enzymatic reaction, histone deacetylase proteins catalyze the removal of acetyl groups on lysine and arginine residues. This dynamic reaction increases the positive charge on histone tails and the binding affinity of regulatory proteins to the DNA sequence; this encourages a more closed chromatin structure and as a consequence represses gene transcription.

Posttranslational modifications of histone tails are integral elements of chromatin structure, transcription, and regulatory behavior, and the methyl-group has received considerable attention in recent years (Figure 1). Histone methylation of lysine and arginine residues provide added complexity to the

| Table 1. Posttranslational Modifications of Histones |
|------------------------|-------------------------|-------------------------|
| Covalent Changes | Histone Residues | Chromosome Function |
| Acetylation | Lysine | Transcription, repair, replication, condensation |
| Methylation | Lysine, m1, m2, or m3 | Transcription, repair |
| Methylation | Arginine, m1 or m2 | Transcription |
| Phosphorylation | Serine, threonine | Transcription, repair, condensation |
| Ubiquitination | Lysine | Transcription, repair |
| Sumoylation | Lysine | Transcription |

| Table 2. Mammalian Histone Acetyltransferase Enzymes |
|------------------------|-------------------------|
| Histone Target | Enzymes Mediating Acetylation |
| H3K9 | Src1 |
| H3K14 | Src1, TAF1, CBP/p300, MOZ/MORF, PACF, GCN5 |
| H3K18 | CBP/p300 |
| H3K23 | CBP |
| H4K5 | Hat1, p300, Tip60, HB01 |
| H4K8 | p300, Tip60, HB01 |
| H4K12 | Tip60, CBP/p300, HB01 |
| H4K16 | Tip60, Mof |
Epigenome and confer distinct nuclear functions. The diversity of chemical variations is also complicated by the number of modifications; for example, in addition to unmodified histones and hyperacetylation, lysine residues can be either mono- (m1), di- (m2), or tri- (m3) methylated (Figure 2). These modifications can exist in different states with recent genome-wide analyses examining histone methylation using chromatin immunopurification (ChIP) strategies coupled with array hybridization or next-generation sequencing having distinguished regions of chromatinized sequences and histone methylation. These recent efforts have revealed that specified histone modifications can be subcategorized; for example, H3K4 trimethylation (H3K4m3) is frequently associated with gene expression and often distributed at transcription start sites. By contrast, gene suppression is accompanied with trimethylation of lysine K9 of histone H3 (H3K9m3), whereas H3K27 trimethylation (H3K27m3) is a prominent feature of euchromatic gene suppression.

Histone methylation patterns are dynamically regulated and are written and erased by methylase and demethylase enzymes with remarkable target and amino acid specificity (Table 3). The striking feature of many of the histone H3 and H4 methyltransferase enzymes identified to date is the conserved SET domain sequence which was first characterized in Drosophila melanogaster and named after Su(var)3-9 (Suppressor of variegation 3 to 9), E(z) (Enhancer of zeste), and trithorax (Trx). The mammalian enzymes responsible for the methylation of H3K4 include Set7/Set9, MLL, and Smyd3 and are associated with transcriptional expression and have been extensively reviewed elsewhere.

Histone Methyltransferases SET the Scene
At the Second Alan Wolffe EMBO Conference on “Chromatin and Epigenetics,” held May 19–22, 2005 at the European Molecular Biology Laboratory in Heidelberg, Germany, we presented some primary findings that transient hyperglycemia caused persistent gene-activating epigenetic changes. Almost 5 years on, and despite some of the advances made in the field, we still know very little of the regulatory mechanism and its role in the expression of key molecules in the diabetic vasculature. What we do recognize are some insights from human primary cell culture models and small animal experiments that associate ambient hyperglycemia with gene-activating events. Further research has expanded our view of the importance of the NF-κB–dependent pathway mediated by Set7 methyltransferase in monocytes, as well as distinguishable regulatory functions in pancreatic islets and cytokine-dependent regulation of NF-κB-p65 activity by lysine methylation. These studies provide critical insights into the regulatory role of the Set7 methyltransferase can strikingly catalyze the
methyl-writing events critical for transcription of histone H3K4–72 and nonhistone proteins such as p53,74 ERα75 and TAF10.76 The function of Set7 in transcriptional regulation is emerging, and these studies now provide some mechanistic insights demonstrating that methyltransferase activity has broad specificity and more importantly show a multiplicity of protein substrates for the lysine residue. It is clear that Set7 involvement in lysine methylation might follow a similar mechanism connecting methylation of determinants involving signal transduction with gene regulatory events.66,77 Thus, Set7 has a primary function in the methylation of lysine residues and this is critical in correctly specifying regulatory roles associated with transcription, as well as establishing histone methylation patterns, namely, H3K4m1 and gene expression events in models of inflammation and diabetes. Furthermore, even though the precise molecular machineries that cooperate with the Set7 enzyme are not well understood and the anticipated “modes of action” discovered to date remain unclear, recent experimental discoveries indicate a common theme with associated with genomic methylation. The Set7 methyltransferase enzyme also regulates the stability of the major maintenance DNA cytosine-5 methyltransferase 1 or DNMT1 (DNA cytosine-5 methyltransferase 1) enzyme, demonstrating functional consequence associated with DNA demethylation.78,79 It is perhaps not difficult to imagine how the Set7 methyl-lysine writer can function in the signaling and transcriptional pathways given the broad specificity of protein substrates, yet, this clearly does not explain how the protein determinants are catalyzed by the Set7 methylase. Important to our research goals and of particular interest to us is to understand the regulatory determinants that segregate and distinguish histone substrate preferences derived from biochemical and mechanistic experiments in primary models of the endothelium and diabetes-induced inflammation (J. Okabe and A. El-Osta, manuscript in preparation). The challenge now is to identify in different cell types how the determinants and the molecular mechanisms that mediate Set7 and associated machineries localize specified proteins and segregate histones in establishing genome-wide methylation and regulatory events.70

Table 3. Mammalian Histone Methyltransferase Enzymes

<table>
<thead>
<tr>
<th>Histone Target</th>
<th>Enzymes Mediating Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3K4</td>
<td>SET7/SET9, MLL, Smyd3</td>
</tr>
<tr>
<td>H3K9</td>
<td>SUV39h1, SUV39h2, G9a, Eu-HMTase1, ESET, SETD1</td>
</tr>
<tr>
<td>H3K27</td>
<td>EZH2</td>
</tr>
<tr>
<td>H3K36</td>
<td>SETD2, NSD1</td>
</tr>
<tr>
<td>H3K79</td>
<td>DOT1L</td>
</tr>
<tr>
<td>H4K20</td>
<td>Pr-SET7/SET8, SUV4-20</td>
</tr>
<tr>
<td>H3R2</td>
<td>CARM1</td>
</tr>
<tr>
<td>H3R26</td>
<td>CARM1</td>
</tr>
<tr>
<td>H4R3</td>
<td>PRMT1</td>
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</table>
Transient Hyperglycemia Causes Sustained Recruitment of Set7 and H3K4 Methylation

We explored the endogenous promoter region of the NF-κB-p65 subunit and identified mono-methylation of histone 3 lysine 4 (H3K4m1). Interestingly, neither di- nor trimethylation at this amino acid residue of histone H3 was altered on the NF-κB-p65 gene sequence in response to glucose. This specific methylation change suggested a potential role for certain methyl-writing enzymes including the Set7 protein. Therefore, we examined recruitment of Set7 to the chromatinized template of NF-κB-p65 demonstrating increased binding of this histone methyltransferase to the NF-κB-p65 sequence. Importantly, the association of other H3K4 methyltransferases such as MLL1 on the promoter of NF-κB-p65 were not specifically changed. Because gene expression occurs as a result of a balance of activating and suppressive gene regulating events, H3K9 methylation is also altered in models of hyperglycemic variability indicating that decreased methylation is inversely associated with gene expression (Figure 3). Indeed, macrophages exposed to high glucose concentrations show reduced H3K9 methylation marks on genes implicated in vascular inflammation. Interestingly, in our own studies using endothelial cells, we observed a dynamic cooperation between H3K4 and H3K9 marks associated with gene activating epigenetic events. Although this does not explain how histone code changes other than H3K4 are conferred, the primary endothelial model of hyperglycemic variability allowed direct investigation of extracellular signals such as glucose in determining other epigenetic changes. We next determined what the role of hyperglycemia on asymmetrical methylation exemplified on H3K4 and H3K9 lysine residues and asked the question; what is the significance of a transient hyperglycemic gradient on histone tail demethylation?

Transient Hyperglycemia Causes Sustained Recruitment of Lysine-Specific Demethylase 1 and H3K9 Demethylation

In a follow-up study, we extended the precedent beyond the H3K4 tail and identified specific changes to H3K9 during transient hyperglycemia and subsequent normal glucose homeostasis. Experimental evidence indicates that antecedent hyperglycemia is associated with persistent upregulation of NF-κB-p65 gene expression and is associated with specific reduction in H3K9 methylation on the promoter region of this gene (Figure 4). Interestingly, transient hyperglycemia causes a sustained reduction in both H3K9 dimethylation (H3K9m2) and H3K9m3 on the NF-κB-p65 promoter, and these events coexist with the recruitment of the lysine-specific demethylase (LSD)1 H3 demethylase on gene promoters. These results suggest that persistent epigenetic-transactions specify asymmetrical lysine methylation of K4 and K9 of histone H3 and strikingly reminiscent of probable multivalent events conferred by hyperglycemia, which will provide critical insights into the relationship of specified histone signatures.

Short- and Long-Term Epigenetic Persistence

Hyperglycemic exposure per se does not carry a set of instructions; instead, we postulate that glucose exposure and more specifically, the continuation of hyperglycemia can signify epigenetic-context dependent signaling that regulates either short- or long-term persistence (Assam El-Osta, unpublished observations, 2008). Since the initial discoveries that glycemic cues can modulate gene-activating epigenetic changes our understanding of histone code changes has challenged many of the original concepts suited for hyperglycemic memory. Indeed, although hyperglycemia can activate gene expression patterns, the reciprocal is also evidenced with suppressed mRNA abundance as well as diverse epigenetic modifications including increased H3K4 and attenuated H3K9 methylation events as adaptive regulatory processes. The general concept that gene–environment interactions not only represent current glycemia, but also include precedent hyperglycemia, which could transmit conserved epigenetic fates that are spatially and temporally consistent with persistent gene activities. This is highlighted by striking plasticity of extracellular signals such as glucose exposure which is dynamically regulating gene activity, indicating that sustained short-term glucose exposure is integrated over time to adapt transcriptional events in pancreatic β-cells. Although the role of hyperglycemia regulating epigenetic determinants or more specifically, methyltransferase function in regulating and specifying histone code fate remains to be determined, there appears to be integrative signaling networks connecting adaptive transcriptional memory mediated by epigenetic cues. Indeed, core pathway components have been identified in converting transient signals into longer lasting-persistent responses that are robustly expressed following stimulus reexposure. For example the incorporation of histone variant H2A.Z into nucleosomes represent memory of epigenetic states associated with transcriptional activity. The coordination of adaptive transcriptional memory mediated by the persistence of epigenetic changes would also explain how the re-exposure of environmental cues such as sugar-specifying signaling cascades determine future cell memories that are integrated by chromatin structure regulating transcriptional events.

Perspectives on Genome-Wide Determination of Epigenetic Signatures

The human genome is almost 2 mln length and is packaged in the nucleus as chromatin that regulates and confers critical metabolic functions to each chromosome. Robustness in chromatin crosstalk and transcriptional coordination allows for the interconnection of epigenetic information to regulate gene sets in a broader program, similar to process networks rather than disconnected-linear regulatory events. For example the coordination of methyl-writing and methyl-erasing enzymes provides a dynamic and robust mechanism of transcriptional control conferred by transient hyperglycemia. Not surprisingly, the genes identified to date have been empirically determined that include members of proinflammatory cytokines. A key challenge in genome-wide analyses is the identification of distinguishable epigenomic patterns that feature transcriptional output and consequence, this means that specifying histone code changes that are associated with gene-regulatory events will require the precise epigenomic mapping if we are to clearly understand the importance of the continued development of diabetic compli-
Hyperglycemia confers gene activating events that are associated with changes in chromatin structure and function. In this schematic, the NF-κB-p65 gene is simplified to show the nucleosome core in yellow consisting of the histones H2A, H2B, H3, and H4 wrapped by the DNA duplex highlighted in blue. The promoter sequence of the NF-κB-p65 gene is contrasted in red to illustrate the changes in chromatin structure that are primary architectural features corresponding with gene regulatory events. Glucose variability, and more specifically transient hyperglycemia, is associated with methyl-writing and methyl-erasing events on histones H3K4 and H3K9, respectively. These molecular events were monitored by ChIP strategies to immunopurify soluble domains associated with methyl-writing and methyl-erasing enzymes that mediate correspondent histone modification and gene expression patterns mediated by transient hyperglycemic exposures. Recent technological advances in genome-wide analyses using ChIP-Seq (ChIP strategies coupled with massive parallel sequencing) now realize this formidable challenge with the potential to map and atlas the epigenome of diabetes. There are several advantages to unbiased determinations of epigenetic signatures. Genome-wide screens provide unprecedented epigenomic-information that are functionally interconnected by intersecting signaling networks with interactive extracellular cues with distinct epigenomic and transcriptional fates. For example, distinguishing the breadth of covalent epigenetic modifications to the histone tail is a formidable challenge, not only because of technology, but also because the list of modifications continues to grow. Empirically identifying histone modification patterns in response to subtle extracellular stimuli such as glucose would be impracticable if considering the epigenomic landscape is more than a linear on/off closed circuit switch, clearly there is significant signaling crosstalk and epigenetic pathways do intersect.

Figure 3. Hyperglycemia causes persistent gene activating changes mediated by chromatin remodeling and the post-translational modification of histones. In this schematic, the NF-κB-p65 gene is simplified to show the nucleosome core in yellow consisting of the histones H2A, H2B, H3, and H4 wrapped by the DNA duplex highlighted in blue. The promoter sequence of the NF-κB-p65 gene is contrasted in red to illustrate the changes in chromatin structure that are primary architectural features corresponding with gene regulatory events. Glucose variability, and more specifically transient hyperglycemia, is associated with methyl-writing and methyl-erasing events on histones H3K4 and H3K9, respectively. These molecular events were monitored by ChIP strategies to immunopurify soluble domains associated with methyl-writing and methyl-erasing enzymes that mediate correspondent histone modification and gene expression patterns mediated by transient hyperglycemic exposures. Recent technological advances in genome-wide analyses using ChIP-Seq (ChIP strategies coupled with massive parallel sequencing) now realize this formidable challenge with the potential to map and atlas the epigenome of diabetes. There are several advantages to unbiased determinations of epigenetic signatures. Genome-wide screens provide unprecedented epigenomic-information that are functionally interconnected by intersecting signaling networks with interactive extracellular cues with distinct epigenomic and transcriptional fates. For example, distinguishing the breadth of covalent epigenetic modifications to the histone tail is a formidable challenge, not only because of technology, but also because the list of modifications continues to grow. Empirically identifying histone modification patterns in response to subtle extracellular stimuli such as glucose would be impracticable if considering the epigenomic landscape is more than a linear on/off closed circuit switch, clearly there is significant signaling crosstalk and epigenetic pathways do intersect.

Figure 4. Persistent epigenetic changes to the chromatinized NF-κB-p65 gene include the recruitment of the Set7 methyl-writing and LSD1 methyl-erasing enzymes that mediate correspondent histone modification and gene expression. Hyperglycemia associated with endothelial dysfunction and alterations in blood vessel growth is the primary cause of vascular complications in diabetes. Furthermore, these vascular complications often persist and may progress despite improved glucose control, possibly as a result of prior episodes of hyperglycemia. In this schematic, we simplify the signaling pathways mediated by hyperglycemia to show some of the key transcriptional events associated with gene activation and the concept of epigenetic persistence. Clinical studies suggest that the injurious effects of exposure to high glucose levels persist for years after better treatment, a phenomenon typically referred to as hyperglycemia memory. Indeed, experimental evidence indicates that short-term memory and the persistent gene expression events are associated with the recruitment of the methyl-writing enzyme Set7 and confer H3K4 methylation on the NF-κB-p65 promoter. In parallel, hyperglycemia is postulated to mediate demethylation of H3K9 by the LSD1 enzyme, demonstrating reciprocal exchange of methyl-writing and methyl-erasing enzymes, recruitment of Pol II, and hyperacetylation events correlating with gene expression.
We are interested in understanding how gene-activating epigenetic changes are regulated by extracellular signals and one project receiving considerable attention is the precise mapping of glucose induced epigenetic signatures (El-Osta et al, manuscript in preparation). The project is aimed to map and intersect epigenetic signatures of diabetes and key experiments using primary aortic cells show distinguishable histone modifications that parallel transcriptional competence. Chromatin immunopurification coupled with next generation-sequencing methodologies distinguish precisely hyperacetylation profiles regulated by hyperglycemia, illustrated and exemplified on human chromosomes (Chr) 3p25.1, 8q11.23, 14q12 and 18q12.1 (Figure 5). Surprisingly, short-term glucose exposure confers dynamic changes in covalent histone modifications that closely correspond to transcriptional output and reveal signatures reminiscent of endothelial cells with proatherogenic features.

**Confering Future Memories**

In summary, it has become increasingly apparent that in both type 1 and type 2 diabetes, there is evidence of ongoing vascular injury as a result of prior transient episodes of poor metabolic control, as demonstrated in both the DCCT/EDIC and UKPDS trials. This has been termed as “metabolic memory” or a legacy effect. With an increasing understanding of epigenetic mechanisms and, in particular, how environmental factors such as glucose can affect the genome, it may now be possible to interrupt epigenetic pathways to promote vascular protection. It is now critical to build on these key findings from in vitro studies, and we can expect the inhibition of specified methyl-writing and methyl-erasing enzymes as targets in attenuating glucose-induced activation of proteins that play critical roles in vascular inflammation. It is anticipated that over the next few years, this hypothesis, which postulates a central role for epigenetic pathways in modulating the development and progression of vascular complications in diabetes, will be greatly strengthened by preclinical studies and clinical investigations that will include molecular and pharmacological approaches to attenuate these glucose-induced changes in chromatin structure and gene function. We are beginning to unravel some of the distinct changes associated with the histone code and the epigenome, which could partly explain why transient elevations of glucose often lead to progressive diabetic vascular complications.

**Conclusions**

Although there are increasingly new therapeutic approaches to improve metabolic control, it remains difficult to achieve near-normal glucose levels in clinical practice. This issue of refractory hyperglycemia with the concomitant impact of hyperglycemic memory implies that over the near to medium term, health systems will continue to be burdened by the social and medical impact of diabetic vascular complications. One innovative strategy to attenuate the burden of complications as a result of prior hyperglycemia is to target the molecular pathways that promote hyperglycemic memory. As our understanding increases in this area, and as these pathways are further elucidated, it is predicted that targeting enzymes implicated in chromatin structure and gene function, such as histone methyltransferases, could be a promising approach. With increased exploration of these targets in various medical contexts, it may be possible in the near future to apply such strategies to preventing and treating diabetic vascular complications.

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**References**


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