

Remodeling is at the heart of chromatin

The heartaches of chromatin

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Chromatin modifications are integral elements of chromosome structure and its function and the vasculature depends on tissue-specific genome regulation for its development. A general concept for the deregulation of chromatin modifications in cardiac and vascular disease is also emerging. The recognition that metabolic memory contributes to disease persistence highlights the benefit of early and aggressive treatment. As for the importance of memory, we do know that good metabolic control delays the onset of long-term diabetic complications. There are striking parallels between the timing of disease and the development of complications. Landmark multicenter clinical trials on diabetes patients have popularized the concept that glucose is also a demonstrable determinant for the development of complications, indicating the prolonged benefit of intensive treatment and the lasting damage of conventional therapy. Each cell type experiences thousands of modifications to the epigenome in response to environmental changes it is exposed to. Therefore, history is neither lost nor forgotten and previous experiences and exposure may form future memories. There is now a strong resurgence in research trying to understand gene-environment interactions and to determine what commits vascular cell types to specific memories. Recent insights show that cardiac gene expression is distinguished by specific chromatin remodeling events and histone modifications that are associated with heart disease.

Legacy of Prior Exposure

The phenomenon of metabolic memory refers to previous exposure to metabolic perturbations that have long-lasting pathophysiological effects, well after the event has dissipated.¹⁻³ For over two decades, the Diabetes Control Complications Trial (DCCT) and the subsequent Epidemiology of Diabetes Interventions and Complications (EDIC) study revealed that a period of suboptimal glycemic control in patients with type 1 diabetes, such as that experienced by those who received conventional therapy with insulin injections, continues to be a risk factor for adverse outcomes, when compared to those who were initially intensively treated, despite the fact that glycemic control has subsequently been similar in the two cohorts for over a decade.³⁻⁷ In more recent work the UKPDS cohort of patients with type 2 diabetes also suggests that the benefits of glucose control can be sustained well beyond the period of the initial trial of intensified treatment.⁸ These studies highlight two important points: first, the development of microvascular and cardiovascular disease takes time and second, that memory exists despite intensive therapy. In addition, animal models of diabetes have shown that the restoration of improved glucose control does not reduce atherosclerosis and the pro-inflammatory impact of hyperglycemia remains and is similar to that seen in mice with persistent hyperglycemia.⁹ Consistent with these findings, diabetic dogs with poor glucose control and then subsequent intensive glycemic control have the same levels of

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retinopathy as those subjected to poor glycemic control for the entire period.¹⁰

With the discovery of hyperglycemic memory in type 1 diabetes, our understanding of metabolic memory and the development of diabetic complications have increased significantly, clearly indicating a significant reduction in the risk of cardiovascular complications.^{6,7} Evidenced in type 1 diabetes is the striking commonality between these epidemiological studies, indicating good glycemic control delays the onset of long-term diabetic complications. The recognition that metabolic memory contributes to this persistence highlights the benefit of early and aggressive hyperglycemic treatment, which reduces the risk of complications, lowering the incidence of retinopathy and nephropathy. The existence of distinct patterns of expression associated with oxidative stress and activating changes in models of hyperglycemic variability have uncovered the role of cellular memory associated with diabetes and inflammation.¹¹ And, although we are beginning to appreciate the risk of persistent complications after euglycemia, we still do not completely understand the molecular determinants associated with hyperglycemic memory.¹²

Distinguishing the Genome with Chromatin Modifications

Emerging evidence suggests the pathogenesis of diabetic complications could be conferred by gene-environment interactions involving chromatin modifications. With the ongoing debate on the benefit of continued monitoring individuals of the DCCT research group¹³ in the late 1980s, there was emerging evidence of the effects of glycemic memory published by Engerman and Kern.¹⁰ Several years later the authors Roy et al. published their findings on the phenomenon of hyperglycemic memory of cultured endothelial cells and postulated that lasting gene-regulating events could be associated with chromatinization.¹⁴ Fast forward several decades later and there has been a resurgence of interest in trying to understand persistent gene activating changes associated with hyperglycemic memory.¹⁵

The view that gene-regulating events are associated with modifications to histone proteins is derived mainly from experiments performed under variable glycemic conditions. Histones are extensively modified by post-translational modifications. Residues on histone H3 tail are the best characterized in model systems including primary culture,⁹ as well as animal¹⁶ and human studies.¹⁷ Although these studies identify the importance of the histone H3 tail, the experimental findings do not explain how the methyllysine writing and erasing enzymes are assembled onto nucleosomes distinguishing chromatin structure and function. It is perhaps not difficult to imagine the importance of transient stimuli such as glucose on intrinsic pathways thought to be associated with persistent transcriptional events.¹⁸ The effectiveness of glucose induced signaling pathways relies on their capacity to tightly control target genes. But what are the true physiological targets, and how broad are transcriptional responses directly epigenetic? Indeed, on the scale that parallels genome-wide approaches defining immediate responses to prior extracellular cues could explain long lasting and persistent changes to gene expression.¹⁹ Contrary to popular belief, sequencing is not the biggest challenge in determining the functional significance of large-scale chromatin modifications. Characterizing distinct transcriptional responses involves analyzing and interpreting large and complex data sets that are critical to build a comprehensive epigenomic picture.²⁰ Considering the emerging data from genome-scale analyses for specific histone modifications allows for correlations between gene expression patterns. We are currently mapping a new route for glucose mediated gene expression changes in a project investigating chromatin modifications as well as genomic methylation, with the aim to atlas and distinguish immediate versus persistent epigenetic signatures (El-Osta et al., manuscript in preparation). As for the importance of specific gene regulatory changes in diabetic vascular disease, several studies have shown the relevance of NF κ B activation as well as the upregulation of chemokines MCP and VCAM, which are key pro-inflammatory cues. Whatever the role

of glucose on transcription, identifying immediate responses and separating these from more persistent genomic changes is the first step to determining roles for specific enzymes involved in writing, erasing or maintaining critical epigenetic marks. Dynamic changes in histone H3 modification such as acetylation could partly explain rapid extracellular responses to hyperglycemia and gene expression. It is more difficult to imagine how hyperacetylation events maintain long or moderate term transcriptional consequences of hyperglycemic memory, unless we consider alternative epigenetic marks. Not so long ago, the conventional view of the genome was the general association that gene suppression was associated with chromatin modifications that were fixed, set and resistant to dynamic change. Recent studies have, however, brought to light key experimental information that is changing the view of genetic rationalism and that methylation changes are indeed flexible and dynamic.²¹⁻²⁴ So, is it important to examine glucose mediated epigenetic changes? Experiments in our laboratory indicate significant local changes in gene-activating epigenetic events conferred by hyperglycemia co-exist with subtle and broad effects throughout the genome. It seems that a fine balance between local versus broad effects with distinct intensities contribute to overall transcriptional responses. The effectiveness of extracellular stimuli conferring a range of signaling intensities determines the capacity to precisely control the expression of target genes with different phenotypes.^{25,26} More than 20 years following the description of hyperglycemic memory, there is now considerable attention to understanding the environmental influences relevant to diabetes associated with the development and persistence of its complications. Now, the focus of research is broadening to determine how the vascular endothelium in context with other cell types perceives these extracellular signals that determine gene-regulating epigenetic changes. Context dependent epigenetic signatures would allow remarkable plasticity and could explain distinct gene expression patterns. As for the invariable stability of gene expression in models of diabetic vascular injury, one of the future challenges

will be to identify systematically the specific role of fatty acids.²⁷ Well studied in development and disease, but of unknown function in diabetes, DNA methylation has been implicated in diabetic vascular disease. The viable agouti (*A^{vy}*) mouse model provided some clues that methylation changes of the metastable *A^{vy}* allele is associated with obesity and diabetes.^{28,29} Although this does not explain how metabolic fuels regulate CpG³⁰ and non-CpG³¹ methylation events, recent evidence implicates this epigenetic change in type 1 diabetes.³²

Remodeling Cardiac Chromatin

Cardiac remodeling in response to pathological hypertrophy results in congestive heart failure and sudden death.^{33,34} The hypertrophic myocardium is characterized by profound changes in gene expression.^{35,36} Our view of the molecular mechanisms regulating the expression of genes in the heart is now emerging with the significance of chemical and structural changes to chromatin. The regulation of gene expression intrinsically depends on multiple histone tail modifications³⁷ that distinguish suppressive and permissive transcriptional events which often converge with changes in DNA methylation³⁸ and ATPase-dependent remodeling activity.³⁹ Precisely how these mechanisms collectively operate in the diseased heart remains poorly understood. Therefore, our views of the molecular mechanisms that direct gene regulatory events are mainly derived from experimental models that are studied independently and in a non-integrated manner. First, the identification of histone modifications associated with enzymes involved in writing and erasing covalent post-translational modifications specify functional consequences that confer specific gene expression signatures.⁴⁰ Second, the realization that enzymes associated with architectural change can contextualize chromatin and confer critical roles associated with transcription highlights the importance of the chromatinized template. Consistent with the aforementioned, there is striking interplay in the chromatin fiber coordinating distinct transcriptional outcomes that are mediated by histone modifications

and ATPase-dependent remodeling.⁴¹ At the inaugural Boston, MA “Cardiac Development and Regeneration” meeting held October 10–11, 2008, we presented some of our primary findings on the context dependent reprogramming of the SWI/SNF complex, specifically allowing Brm and Brg1 to differentially serve as co-regulatory determinants at promoters of the hypertrophied heart. Recent interest in this area now shows that cardiac and chromatin remodeling is associated with the expression of the myosin heavy chain with one of the identified SWI/SNF determinants. Chromatin remodeling by Brg1 underlies heart muscle development and was associated with the switch of α -to β -MHC gene expression.⁴² Chromatin has many ways to regulate the expression of target genes and, so far, it has been difficult to monitor these specific events in ventricular tissues. With the left and right ventricular chambers of the heart distinguishable in physiology and gene expression, it appears that the function of histone modifications also follows a similar pattern of distinction.⁴³ The precise nature of nucleosome recognition by SWI/SNF proteins remains poorly understood and further studies are necessary to directly test the roles of other epigenomic modifications in discriminating specific sequences and the regulation of gene expression. Determining the regulatory roles for SWI/SNF remains a significant challenge in understanding the remodeling events associated with cardiac development and disease.^{44,45} Evidence now indicates the reversibility of cardiac events and parameters associated with hypertrophy by histone deacetylase inhibition, suggesting dynamic assembly of determinants on the chromatinized template is associated with restoring gene expression patterns.⁴⁶ In the left ventricle, the experimental evidence implies the distinguishable and opposing roles of Brm and Brg1 in the hypertrophied heart. This was exemplified with the specific expression of ANP (*Nppa*), BNP (*Nppb*) and β -MHC (*Myh7*), which were inversely correlated with α -MHC (*Myh6*) and *Serca2a* (*Atp2a2*) genes. These gene expression changes were associated with contextualized binding of SWI/SNF determinants on promoters regulating the fetal gene expression in

heart disease. Distinct binding on these genes distinguishes the suppressive Brg1 associated HDAC2 complex from that of Brm, which is enriched on activated genes and parallels CBP/p300 binding in hypertrophy. The histone deacetylase inhibitor, trichostatin A, prevented left ventricular hypertrophy, which was also associated with contextual binding of SWI/SNF determinants. The emerging experimental evidence now indicates that the SWI/SNF complex serves as a coregulator of gene expression in the development of pathological cardiac hypertrophy. Whether the Brm or Brg1 determinants, like the other members of the SWI/SNF family, serve in establishing and maintaining suppressive and permissive chromatin structures as marks of epigenomic persistence in the diseased heart remain an interesting area for future investigation.

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