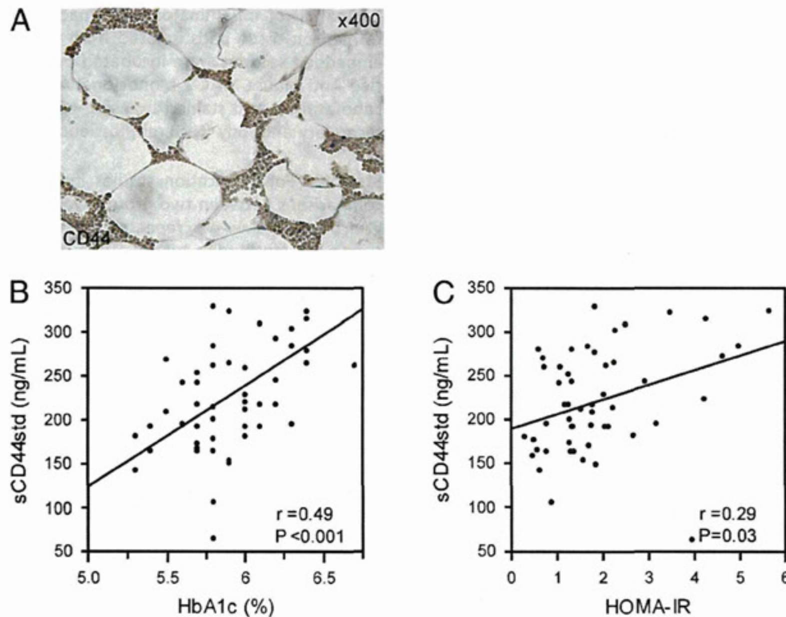

Fig. 5. CD44 functional experiments in human subjects. (*A*) Paraffin-embedded omental adipose tissue from an obese woman [age (yr); 57, BMI (kg/m^2); 36.9] was analyzed for CD44 immunoreactivity. (*B* and *C*) The correlation between serum levels of standard soluble CD44 (sCD44std) and either an index of glycemic control, HbA1c (*B*), or an index of insulin resistance, HOMA-IR (*C*), determined by using a linear regression model estimated with minimal square method in human subjects [n = 55: mean ± SD age (yr), 60.3 ± 15; BMI (kg/m^2), 23.2 ± 4.3; fasting plasma glucose (mg/dL), 109 ± 13; fasting plasma insulin (μU/mL), 6.22 ± 3.84; HbA1c (%), 5.9 ± 0.34].

the soluble form of CD44 correlate with increasing hyperglycemia and insulin resistance in humans. Although the expression of CD44 in macrophages and T cells in obese adipose tissue has been reported (36, 37), this study directly addresses the functional role of CD44 in adipose tissue inflammation. The findings that systemic glucose intolerance is ameliorated by CD44 depletion and blockade by using CD44 mAb strongly suggest that CD44-dependent adipose inflammation has an impact on systemic metabolism. However, further studies are needed to determine which immune-cell population primarily expresses CD44 receptor (macrophages or T cells?) and how the CD44 molecules initiate and maintain immune-cell infiltration into adipose tissue (through the SPP1 signals?). Even so, these results indicate the significance of CD44 immune-receptor as a possible therapeutic target for T2D and a unique biomarker for insulin resistance.

In conclusion, we discovered that an immune-cell receptor gene, *CD44*, is pathogenetically implicated in the development of adipose tissue inflammation and insulin resistance, using a data-driven candidate gene approach by using an eGWAS method of integrating >1,000 publicly available genome-wide functional microarrays related to T2D. Application of the eGWAS methodology to publicly available data can yield promising candidate genes that are differentially expressed in T2D-relevant tissues, independently of knowledge about insulin signaling, glucose, or lipid metabolism. We suggest that a data-driven approach can enable investigators to consider glucose homeostasis phenotypes from a different point of view and notice new pathways that could be involved in the development of T2D. Although GWAS and other genetic analyses will continue as the method of choice for the next few years, an eGWAS approach could complement these studies to yield additional pathogenetically important genes for many other complex diseases by using this integrated data-driven approach.

Materials and Methods

See *SI Materials and Methods* for further descriptions.

eGWAS. All T2D-related genome-wide microarray experiments used for this study were collected from three public data sources: the NCBI Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo), the Diabetes Genome Anatomy Project (DGAP; www.diabetesgenome.org), and the Nuclear Receptor Signaling Atlas (NURSA; www.nursa.org). There were a total of 1,175 samples (591 T2D cases and 584 controls) in 130 independent datasets. To estimate differences between groups of samples from diabetic subjects and groups representing control, raw postquantitation microarray data were reanalyzed by using Significance Analysis of Microarrays software (SAM) (38). For each gene in every microarray experiment with three or more samples in each group, we calculated a *d* score (d_i), which denotes the standardized change in gene expression:

$$d_i = \frac{\bar{x}_{i-t2d} - \bar{x}_{i-control}}{S_i + S_0},$$

where $\bar{x}_{i-t2d}$ is the mean expression level of gene i in group T2D, $\bar{x}_{i-control}$ is the mean expression level of gene i in group control, S_i is the SD for the numerator calculation, and S_0 is a small positive constant. We considered genes to be significantly dysregulated with either an absolute value of the *d* score ≥2 or a fold change ≥2 between controls and cases. We then converted all probe identifiers across the various microarray platforms for mouse, rat, and human to the latest human Entrez Gene identifiers by using our published AILUN system (39). Gene expression profiles were assigned in our eGWAS database according to the standardized (human) Entrez Gene ID. There were 24,898 genes in the database in total. For every one of the 24,898 genes, we counted the observed number of microarray experiments in which each gene was significantly dysregulated. We then calculated *P* values from the number of positive/negative experiments for every one of genes and sum of the number of positive/negative experiments for all other genes, using a 2 × 2 χ^2 analysis (Fig. 1) or a Fisher's exact test as an alternative (Fig. S3), and ranked all of the genes according to their *P* values [$-\log_{10}(P)$]. A third method, Liptak–Stouffer's weighted Z-method (40), provided additional support for *CD44* (Fig. S4).

Animal Experiments. Mice for breeding, C57BL/6J wild-type (diabetes-prone) and *CD44*-deficient mice backcrossed to C57BL/6J for at least 10 generations (B6.Cg-*Cd44^{tm1Hbg}*/J), were obtained from The Jackson Laboratory. Mice were housed in a barrier facility under specific pathogen-free conditions. The Animal Care and Use Committee of Kitasato University approved all animal experiments.

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Human Studies. Venous peripheral blood samples were collected from human subjects who went through a 75 g oral glucose tolerance test after an overnight fast [$n = 55$: sex (M/F); 36/19]. HbA1c was measured in Japan Diabetes Society (JDS)-HbA1c units and then converted to National Glycohemoglobin Standardization Program (NGSP) levels by the formula HbA1c (%) (NGSP) = HbA1c (JDS) (%) + 0.4% (41). We then calculated homeostasis model assessment as an index of insulin resistance [HOMA-IR = fasting plasma insulin (μU/mL) × fasting plasma glucose (mg/dL)/405] as described (42). Serum sCD44std (standard soluble CD44) and SPP1 concentrations were determined by using a quantitative ELISA technique (sCD44std ELISA, Bender MedSystems; Human Osteopontin Quantikine ELISA, R&D Systems).

Informed consent was obtained from all of the subjects enrolled in this study, and the protocol was approved by the ethics committee of the University of Tokyo.

Immunohistochemistry. For histological analysis of CD44 expression in adipose tissue, EWAT was removed from mouse models, and omental adipose tissue obtained from consented donors undergoing elective gastric bypass surgery (lot no. OM020304B) was purchased from Zen-Bio. Formalin-fixed paraffin-embedded sections were stained with mouse monoclonal antibody against CD44 at 1:50 dilution (DF1485/sc-7297; Santa Cruz Biotechnology), followed by reactions with anti-mouse immunoglobulins-HRP, and anti-fluorescein-HRP. 3,3′-Diaminobenzidine (DAB) was used as a chromogen.

Analysis of inflammatory cell (macrophage) content in EWAT was performed on tissue pads isolated from model mice. Formalin-fixed paraffin-embedded sections were incubated overnight with primary antibody: Purified Anti Mouse MAC-2 Monoclonal Antibody (CL8942AP, 1:100; Cedarlane Laboratories) and stained by using Histofine Simple Stain Mouse MAX-PO secondary antibody (Nichirei Biosciences) with a DAB solution.

Statistics. For verification studies in mouse models and human subjects, comparisons between two groups were performed by using the two-tailed Welch's *t* test. Two-way repeated measures ANOVA was used to examine the treatment (antibodies type) × time interaction on blood glucose changes from baseline. *P* values of <0.05 were considered significant. All experimental data are represented as mean ± SE unless otherwise noted.

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GENETICS (T FRAYLING, SECTION EDITOR)

The Epigenome and Its Role in Diabetes

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Abstract Both genetic and environmental factors play critical roles in the development of diabetes. Epidemiological evidence and data from clinical studies suggest the persistence of a "metabolic memory" of past exposures to environmental factors or glycemic control. Epigenetic mechanisms are regarded as one of the likeliest candidates underlying these phenomena. On the other hand, owing to the recent elucidation of mechanisms that erase epigenetic marks, it has gradually become recognized that epigenetic regulation is a more dynamic process than previously thought. A technological breakthrough in epigenome research in the past decade was the development of high-throughput sequencing. This new technology lets us investigate the epigenome in a global and comprehensive manner, and provides previously unrecognized findings and insights. This review presents an overview of the recent progress in our understanding of epigenetic regulation in type 1 and type 2 diabetes research.

Keywords Epigenetics · Epigenomics · Epigenome · Next generation sequencer · High throughput sequencing · ChIP-seq · FAIRE-seq · Histone methylation · Histone acetylation · DNA methylation · Diabetes · Metabolic memory · Legacy effect · Adipocytes · Pancreatic islet · Liver · Muscle · Vascular cells

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Introduction

Genomic DNA in eukaryotes is tightly packed in the nucleus of the cell in combination with histone proteins, forming a highly-organized structure called "chromatin." Regulation of chromatin structure is critical in transcriptional regulation of gene expression. Posttranslational modification of the chromatin such as DNA methylation, histone acetylation, and methylation is known to be an essential component of epigenetic regulation. There is an increasing awareness that epigenetic processes play a critical role in diabetes and metabolic disorders. Here, we review the definition of epigenetics and epigenomics with an emphasis on both stable and dynamic nature of epigenetics. We then discuss their role as a link between environmental factors and the development of diabetes, plus new findings and emerging concepts from genome-wide epigenomics analyses and recent advances in our understanding of epigenetic regulation in various organs related to diabetes; finally, we review the potential of epigenetic regulation as a target of drug development for the treatment of diabetes.

Epigenetics and Epigenomics

In the 1940s, Conrad Waddington first created the term "epigenetics," which was originally defined as "the unfolding of the genetic program for development" [1]. In 1975, DNA methylation was first suggested as the principal factor behind the hypothesis that Waddington had proposed [1, 2]. In mammals, DNA methylation occurs on cytosine residues of CpG dinucleotides, and the methylation of DNA closely correlates with transcriptional repression [3, 4]. The genomic patterns of cytosine methylation are regulated by both de novo and maintenance DNA methyltransferases (DNMTs). Since the maintenance DNMTs add methyl

groups to hemi-methylated DNA during DNA replication, enabling the inheritance of the pattern of DNA methylation on mitosis, DNA methylation is considered the best-characterized chromatin modification that provides a stable, and critical component of epigenetic regulation. Today, epigenetics is described as the study of stable yet reversible molecular mechanisms, including DNA methylation and covalent modifications of histone tails such as acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation, which lead to a given phenotype without a change in genotype [2, 5]. Global rather than local investigation of "epigenetics" is referred to as "epigenomics" in analogy with "genomics" in relation to "genetics."

DNA Methylation

DNA methylation occurs almost exclusively at the cytosine of CpG dinucleotides, which tend to cluster in regions called "CpG islands" [6]. One definition of CpG islands associated with the 5' regions of genes is DNA regions greater than 500 bp with a GC content greater than 55 % and an observed-to-expected CpG ratio of 65 % [7]. CpG dinucleotides are very uncommon in mammalian genomes (~1 %) because methylated C residues spontaneously de-aminate to form T residues. The CpG sequence in CpG islands usually remains unmethylated when the entire genome undergoes de novo methylation, although some become methylated in a tissue-specific manner during development [8]. DNA methylation is generally associated with suppression of gene expression. Methylated cytosines are recognized and bound by the methyl-CpG-binding domain (MBD) protein MeCP2, which directly recruits histone deacetylase 1 (HDAC1) and Sin3A to mediate inhibitory action on gene expression [4]. DNA methylation is established and maintained largely by DNA methyltransferase (DNMT) 1, DNMT3a, and DNMT3b [5]. DNA methylation is essential for mammalian development: loss of *Dnmt1* results in embryonic lethality around embryonic day (E) 8.5–9 in mice [9]. *Dnmt3b* mutant mice do not develop to term, showing multiple developmental defects. Homozygous *Dnmt3a* knockout mice can develop to term but die within a month after birth [10]. DNA methylation also plays an essential role in many cellular processes including silencing of repetitive and centromeric sequences and X chromosome inactivation in female mammals [3].

Post-Translational Histone Modification

Genomic DNA and specific proteins form a structure called "chromatin" in eukaryotes in order to be packed in a limited volume of the cell (Fig. 1). The basic unit of the chromatin structure is a nucleosome that consists of approximately 147 base pairs of DNA wrapped in 1.65 turns around a histone octamer consisting of 2 copies, each, of the core histones H2A, H2B, H3, and H4. Nucleosomes are connected by stretches of linker DNA (on average ~50 bp), which are bound by histone H1 [6]. Accumulated evidence has suggested that post-translational modification of histones plays an essential role in regulation of chromatin structure and gene expression [11]. Such modification includes acetylation, methylation, phosphorylation, ubiquitination and glycosylation [12], among which histone acetylation and methylation have been intensively investigated.

Histone acetylation was first described in 1964. Acetylation of lysine residues on the histone tails is mediated by enzymes called "histone acetyltransferases" that catalyze the transfer of an acetyl group to the ε-amino group of lysine side chains on the histone protein by using acetyl-CoA as a cofactor. Counter-reaction (deacetylation) is known to be mediated by enzymes called "histone deacetylases" (HDACs). Histone acetylation neutralizes the positive charge on lysine, thus reducing the affinity of the histone tail that protrudes from the nucleosome core of DNA, allowing chromatin to form a more relaxed structure closely associated with gene activation [11].

In contrast, the significance of histone methylation in gene regulation has been gradually elucidated only over the past few decades. Lysine residues on histone tails can be monomethylated, dimethylated, or trimethylated. Arginine residues are also subject to methylation [11, 13]. Methylation does not change the charged state of a lysine or arginine residue and does not appear to directly alter chromatin structure [11]. Instead, methylated lysine or arginine residues and surrounding amino acids serve as sites recognized by the histone "reader" proteins that recruit other regulatory proteins and ultimately affect the chromatin structure and gene expression. For example, methylated histone H3 lysine 4 is recognized and bound by the CHD1 chromodomain, which recruits an activating complex, leading to gene activation, whereas methylated histone H3 lysine 9 and histone H3 lysine 27 are bound by heterochromatin protein 1 (HP1) and Polycomb group (PcG) proteins, respectively, which mediate chromatin compaction, leading to gene inactivation [14]. In contrast to histone acetylation, whether histone methylation is associated with either activation or repression depends on the position of the amino acid to be methylated [11]. There are increasing numbers of histone-modifying enzymes that catalyze addition and removal of the methyl group and the acetyl group to and from histones identified in the past decade. A systematic nomenclature for the enzymes has been proposed to avoid confusion [15].

In addition to DNA methylation, histone modification also composes a layer of stable epigenetic information [14]. Compelling evidence supports the stability of specific

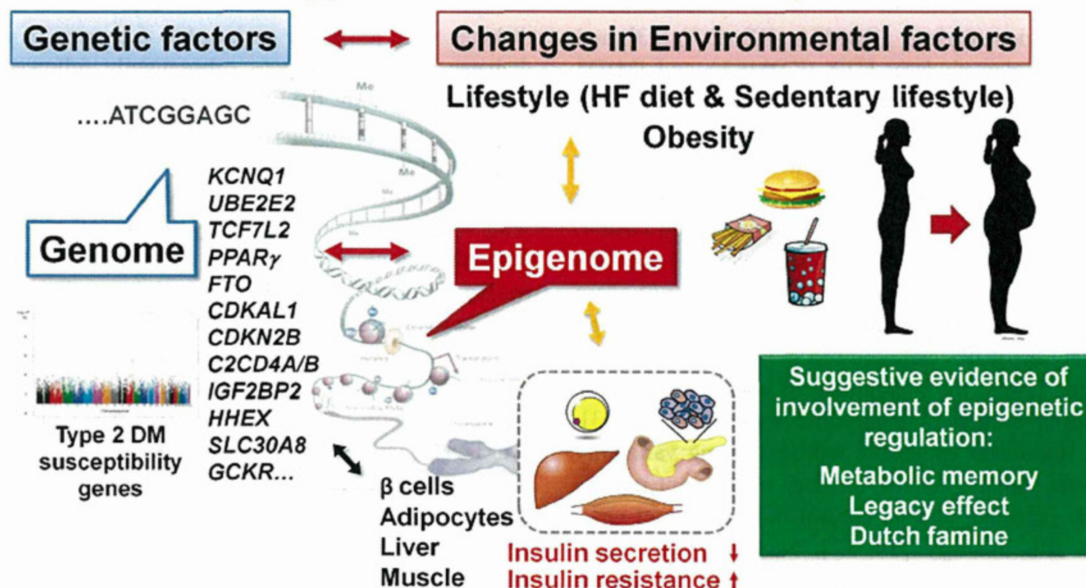

Fig. 1 Involvement of epigenetic regulation in type 2 diabetes and obesity. Both genetic predisposition and environmental factors play critical roles in the development of type 2 diabetes. Environmental factors can impact the epigenome of human subjects. Epigenetic regulation is implicated in mechanisms underlying phenomena such as the legacy effect, that is, the long-lasting beneficial effect of intensive therapy of type 1 and type 2 diabetes on development of diabetic complications [44–47], and in the influence of intrauterine undernutrition in the Dutch famine on the development of type 2 diabetes and obesity in later stage in life [48]. (Adapted with permission from: Task E, Board SA. Moving AHEAD with an international human epigenome project. Nature. 2008;454:711–5) [71]; and (Adapted with permission from: Yamauchi T, Hara K, Maeda S, et al. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat Genet. 2010;42:864–8) [133]

histone modifications including H3K4 and H3K27 methylation, which are catalyzed by PcG and Trithorax-group protein complexes, respectively [14, 16]. Chromatin immunoprecipitation (ChIP) analysis revealed that many promoters of developmental genes are associated with both H3K4me3 and H3K27me3 active H3K4me3 and such genomic regions, which is referred to as "bivalent domain" [14, 17]. Bivalent modification is proposed to represent the "poised" yet unexpressed status of developmental genes in undifferentiated stem cells and will be turned on or off depending on differentiation cues. In this regard, the stability of H3K4me3 and H3K27me3 may serve as one of the fundamental mechanisms underlying cellular memory of lineage specification.

Dynamic Nature of Epigenetics

Although epigenetic marks, particularly DNA methylation, have been considered stable and persistent, it is becoming increasingly recognized that epigenetic marks are more reversible and more dynamically regulated than originally thought; this is largely due to better understanding of the DNA and histone demethylation processes. There are 2 types of mechanisms for DNA demethylation: active and passive. While passive DNA demethylation occurs when DNA maintenance methylation is blocked during DNA replication in mitosis, active DNA demethylation involves enzymatic replacement of 5-methylcytosine with C. Active DNA demethylation is implicated in rapid genome-wide paternal genome demethylation after fertilization, and also in loci-specific active DNA demethylation in somatic cells in response to external signals. Examples of the latter include acute DNA demethylation in the interleukin-2 promoter-enhancer region in T lymphocytes after twenty-minute stimulation [18], the brain-derived neurotrophic factor gene promoter in post-mitotic neurons [19], and the peroxisome proliferator-activated receptor gamma, coactivator 1 α (PGC-1α) promoter after acute exercise [20••]. Many enzymes, modes and mechanisms involved in active DNA demethylation have been proposed and intensively reviewed by Wu et al. [21]. Like DNA methylation, histone methylation had been thought irreversible, but thanks to recent discoveries of various specific histone demethylases, it has been gradually recognized that histone methylation is a more dynamic and reversible process. The first enzyme

identified as a histone lysine-specific demethylase was the amine oxidase LSD1 (KDM1 in the new nomenclature) [22], followed by a series of JmjC demethylases such as H3K9-specific demethylase, JHDM2A (KDM3A) [23], and H3K27-specific demethylases UTX (KDM6A), and JMJD3 (KDM6B) [24–28]. Another evidence that supports the dynamic and plastic nature of epigenetic marks came from the discovery of induced pluripotent stem cells (iPS) [29]. As described in their original paper, Takahashi and Yamanaka created iPS cells from somatic cells by introducing *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*—the "Yamanaka factors"—and they reported that the promoters of *Nanog* and *Fbx15* were demethylated after reprogramming [29]. Genome-wide epigenetic analyses of iPS cells showed global reprogramming at epigenetic levels: the reprogrammed epigenetic status is highly similar to that of embryonic stem cells [30–33]. However, significant epigenetic reprogramming variability due to either somatic cellular memory or aberrant reprogramming is reported, and its control is one of vital challenges in iPS biology [34–37]. Nevertheless, these phenomena collectively highlight the dynamic and plastic nature of epigenomic marks.

Epigenetics: Possible Link Between Environmental Factors and the Development of Type 2 Diabetes

Both genetic predisposition and environmental factors play critical roles in the development of metabolic diseases including obesity and type 2 diabetes (Fig. 1) [38]. Since epigenetic mechanisms can integrate environmental cues at the cellular level, they rank among the promising candidates that link environmental factors and the development of type 2 diabetes [38]. The impact of environment on the epigenome of human subjects was elegantly shown in a recent study by Fraga et al. [39, 40]. They investigated global and locus-specific differences in DNA methylation and histone acetylation in monozygotic twins of various ages, and observed that older identical twin pairs show substantial epigenetic variations while young identical twin pairs are nearly identical in their epigenetic marks [39, 41]. This observation clearly indicates that the environment can alter the epigenetic status of individuals during their lifetime, which is likely to explain the difference in susceptibility of monozygotic twins to various diseases.

In the United Kingdom Prospective Diabetes Study (UKPDS), a randomized controlled trial, intensive diabetes treatment substantially decreased the risk of microvascular complications in type 2 diabetes patients [42]. Interestingly, during 10 years of post-trial follow-up, despite an early loss of glycemic differences, a continued reduction in microvascular risk and emergent risk reductions for myocardial infarction and death from any cause were observed—the "legacy effect" [43]. Similar phenomenon also appears to be active in type 1 diabetes: In the Diabetes Control and Complications Trial (DCCT), 1441 persons with type 1 diabetes were randomly assigned to 6.5 years of intensive diabetes therapy. This was subsequently followed by the observational study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, which revealed a long-term protection from macro- and micro-vascular events among patients treated early in the course of type 1 diabetes with intensive diabetes therapy in DCCT [44–47]. The pathophysiological mechanisms responsible for this long-lasting effect are unclear. But epigenetic regulation is one of leading hypotheses explaining this effect.

Epidemiological studies of human individuals who were prenatally exposed to famine during the "Dutch Hunger Winter" (a short-term famine in 1944–1945 during World War II) also imply that epigenetic memory is involved in the development of metabolic diseases such as type 2 diabetes and obesity. Individuals so exposed exhibited not only alteration in birth-weight and body conformation at birth, but also had a greater incidence of obesity, type 2 diabetes and dyslipidemia [48]. This phenomenon is sometimes described as "developmental programming" [49]. Indeed, involvement of epigenetics was demonstrated by a number of studies. Heijmans et al. showed that such individuals possess less DNA methylation of imprinted IGF2 gene compared with their unexposed, same-sex siblings after 60 years [50]. This association was particularly observed when the exposure occurred in the periconceptional period, suggesting that very early mammalian development is a crucial period for establishing epigenetic marks [50]. On the other hand, Godfrey et al. investigated epigenetic status of genomic DNA derived from the umbilical cord at birth in 2 large-scale cohorts and prospectively followed up individuals. They found that the degree of methylation of the promoter region of RXRα correlates with body adiposity at ages 6 and 9 years [51•]. In another prospective study by Toperoff et al. of non-symptomatic individuals with a mean age of 30 years, degree of methylation of a CpG site of FTO in blood samples had a small but significant increased risk of impaired glucose tolerance appearing by a mean age of 43 years [52].

Many animal studies also reinforce the involvement of an epigenetic mechanism linking the environment and alteration in gene expression leading to disease phenotype. Variable yellow agouti (A^{vy}) mice harbor a transposable element in the *agouti* gene [53]. The allelic expression of the *agouti* gene is correlated with the epigenetic status of a transposon that is associated with the promoter region of the gene. When expressed, the *agouti* gene causes yellow fur, type 2 diabetes, obesity and tumorigenesis. Maternal dietary methyl-donor supplementation of these mice with folic acid, vitamin B12, choline and betaine resulted in dramatic

alteration of the degree of methylation of the region, fur color and metabolic phenotype [2, 54]. Park et al. investigated in a rodent model of intrauterine growth retardation epigenetic alteration of the promoter region of *Pdx1*, a critical regulator of pancreatic β cell development, and demonstrated sequential epigenetic alteration that involved deacetylation histones H3 and H4, demethylation of histone H3 lysine 4 (H3K4), methylation of H3K9 followed by methylation of the CpG island in the proximal promoter, causing repression of Pdx1 expression and subsequent development of type 2 diabetes in adulthood [55]. Similarly, Sandovic et al. reported that a low-protein maternal diet in rats led to an epigenetic and chromatin conformation change in the *Hnf4a* locus of the offspring [56].

Transgenerational Epigenetic Inheritance

In some cases, metabolic reprogramming appears to be passed through generations, which is generally termed "transgenerational epigenetic inheritance" [57]. Recent reports suggested transgenerational epigenetic inheritance of longevity in *C. elegans* [58] and transgenerational inheritance of stress-induced epigenetic change in *D. melanogaster* [59]. In rodent models, Jimenez-Chillaron et al. examined intercrossed female and male F1 control: mice underwent undernutrition in utero during late gestation and showed reduced birth weight, IGT and obesity in F2 second-generation offspring [60]. Dunn et al. demonstrated that the influence of maternal high-fat diet on body weight and insulin sensitivity affects even F3 offspring [61]. Recent reports suggested not only maternal nutrition but also paternal nutrition have an impact on the health outcomes of adult offspring. Ng et al. demonstrated in a rat model that paternal exposure to high-fat diet resulted in β-cell dysfunction in rat F1 female offspring [62••]. Independently, Carone et al. also demonstrated that paternal low-protein diet resulted in altered hepatic expression of genes involved in lipid homeostasis and altered methylation changes including a likely enhancer of the key lipid regulator PPARα [63••]. It is speculated that several mechanisms, including DNA methylation and regulatory RNA species, are involved in such "transgenerational epigenetic inheritance via the gametes."

Mechanisms Linking Extracellular Signals and the Epigenome

From a mechanistic standpoint, how do cells translate environmental cues to the epigenome? Representative extracellular signals are cytokines, growth factors, and hormones. These factors typically bind to their receptors, causing subsequent cascades of intracellular signaling and alteration of cellular functions. One of the major outputs of signaling molecules is transcriptional regulation of genes by remodeling the epigenome by transcription factors recruiting chromatin modifying factors in a locus-specific manner. RNA-mediated deposition of epigenetic signals has been another intensive research area. Non-coding RNAs (ncRNAs) such as long ncRNAs and PIWI-interacting RNAs are examples, and were reviewed by Bonasio et al. [16]. A recently emerging alternative mechanism is that cellular nutrient levels and metabolism alter levels of substrates and co-factors of chromatin-modifying enzymes, causing change in epigenetic status [64, 65]. Wellen et al. demonstrated that ATP-citrate lyase, the enzyme that converts glucose-derived citrate into acetyl-CoA, is required for histone acetylation and gene expression in response to growth factor stimulation and differentiation in mammalian cells [66]. Cai et al. recently showed that oscillating cellular levels of acetyl-CoA, the donor to histone acetylation reaction, play a critical role in histone acetylation of genes important for growth in budding-yeasts transiently administered metabolites [67]. S-adenosyl methionine is made from adenosine triphosphate (ATP) and methionine, and is an important substrate involved in methyl group transfer including methylation of DNA and histones such as DNMTs and HMTs. α-Ketoglutarate, the cofactor for JHDM histone demethylases and TET family DNA demethylases, is a TCA cycle intermediate. LSD1 histone demethylases require flavin adenine dinucleotide (FAD) as a co-factor. Other examples of metabolites working as cofactors and substrates include NAD^+ for Sirtuin histone/protein deacetylases, AMP/ATP ratio for AMP-activated kinase, and GlcNAc for O-GlcNAc transferase [67].

Genome-Wide Approach for Analyses of Epigenomics of Diabetes

One of the landmark technological advances in genome science in the past decade is the development of next generation sequencing (NGS) technology such as the Illumina Solexa Genome Analyzer, Roche 454 Genome Sequencer and Applied Biosystems SOLiD platforms. DNA sequencing cost dramatically reduced 14,000 fold from 1999 to 2009 [68]. NGS technologies have revolutionized how we study the epigenome and transcriptional regulation of genes. ChIP, using specific antibodies followed by high-throughput sequencing (ChIP-seq), allows genome-wide mapping of binding sites of transcription factors and genomic regions of specific chromatin modifications. Several methods, such as MeDIP-seq and reduced representation bisulfate sequencing, are employed to map DNA methylation on a global scale. The DNase I hypersensitivity assay and Formaldehyde-Assisted Isolation of Regulatory Elements

coupled with high-throughput sequencing (DNase-seq and FAIRE-seq, respectively) detect regions with open chromatin structure. These new approaches provide novel insights never before gained and broaden our understanding of epigenetic regulation of gene expression. Collaborative efforts to generate and analyze epigenetic maps of the human genome (reference epigenome) in various types of cells have been made; these include the ENCODE project [69], the US National Institutes of Health Roadmap Epigenomics Program [70] and the Alliance for the Human Epigenome and Disease [71]. Of note, the ENCODE consortium recently published 1,640 genome-wide epigenome data sets prepared from 147 cell types providing a series of papers in Nature and other journals [72••]. Publically accessible resources of epigenetic maps generated through these efforts are expected to enormously contribute to the entire scientific field. In this section, we will review recent publications of genome-wide investigation of epigenome in various cell types relating to diabetes.

1. Adipocytes

Adipose tissue plays the central role in the development of type 2 diabetes and metabolic syndrome [73]. Since adipocyte differentiation is closely linked to systemic glucose and lipid homeostasis and is one of the best-characterized models of cellular differentiation [74], it has been a subject of intense genome-wide analyses of the epigenome. One of novel findings revealed by genome-wide identification of binding sites of the master adipogenic regulator peroxisome proliferator-activated receptor (PPAR) γ in murine 3T3-L1 adipocyte cell line was that PPARγ co-localized with the other master regulator, CCAAT/enhancer-binding protein α (C/EBPα) on a global scale and cooperatively regulate adipocyte differentiation [75, 76]. More genome-wide time-course analysis revealed that transient binding of glucocorticoid receptor (GR) and C/EBPβ in the early phase creates an epigenomic transition state that is necessary for subsequently triggering the adipogenic program [77]. A study by Siersbæk et al. revealed extensive chromatin remodeling and establishment of transcription factor "hotspots" where multiple early transcription factors cooperatively bind in the early phase of adipogenesis [78•]. Transcription factor binding in adjacent binding on a genome-wide scale, referred to as "co-localization," is one of the emerging concepts in genome-wide analysis of transcription-factor binding sites. Whereas PPARγ co-localizes with C/EBPα to cooperatively regulate adipogenic gene transcription in adipocytes, PPARγ co-localizes with the hematopoietic transcription factor PU.1 near a distinct set of immune genes in macrophages, suggesting that a cell-type-specific co-localizing partner is a critical determinant of cell-type-specific binding sites and function of PPARγ [79•]. Eguchi et al. conducted qPCR-based high-throughput DNase hypersensitivity analysis of 27 adipocyte-specific genes and identified 268 such regions in mouse 3T3-L1 adipocytes [80]. Employing a computational motif analysis, they identified IRF transcription factors as new regulators of adipocyte differentiation. Mikkelsen et al. conducted a comparative genome-wide epigenomic analysis of both murine and human adipocytes by using ChIP-seq, demonstrating that the location of PPARγ binding sites differ in 2 species even near orthologous loci with similar expression patterns [81•]. This difference appears to be created through evolutional turnover of the transcription factor motif. They performed motif analysis and identified PLZF and SRF as negative regulators of adipogenesis [81•]. We mapped adipocyte-specific regulatory elements in mouse 3T3-L1 adipocytes by employing FAIRE-seq, which detects open chromatin genomic regions [82•]. Non-promoter FAIRE peaks were characterized by H3K4me1+/me3-, the signature of enhancers, and showed dynamic change during differentiation while the promoter FAIRE peaks were relatively constant. Genes highly up-regulated during differentiation were typically associated with multiple clustered adipocyte-specific FAIRE peaks, elucidating the functional importance of "multiple" distal enhancers for optimal adipogenic gene expression. The computational motif analysis of the adipocytes-specific FAIRE peaks led to identification of nuclear family I transcription factors as a novel regulator of adipocyte differentiation [82•]. Together, these studies demonstrate the utility and advantage of genome-wide approaches in providing a global view of cell-type-specific regulatory elements in the genome and in identifying transcriptional regulators of adipocyte differentiation in an unbiased manner.

The Wnt/β-catenin pathway is inhibitory to adipogenesis. Okamura et al. performed ChIP followed by microarray analysis (ChIP-on-chip) for β-catenin in adipocytes and demonstrated that nuclear receptor COUP-TFII is a critical mediator of β-catenin's inhibitory effect on mouse 3T3-L1 adipocytes [83]. Wakabayashi et al. performed ChIP-on-chip analysis of PPARγ binding sites and demonstrated that PPARγ induces expression of the histone H4 Lys 20 (H4K20) monomethyltransferase PR-Set7/Setd8 (KMT5A) and that this regulation is part of the mechanism by which PPARγ regulates its target genes [84]. Li M et al. recently sampled eight variant adipose and 2 distinct skeletal muscle tissues from 3 pig breeds, generated an atlas of DNA methylomes, and demonstrated that differentially methylated regions in promoters are highly associated with obesity development by means of expression repression of both known obesity-related genes and novel genes [85].

2. Skeletal Muscle

Blow et al. performed ChIP-seq for the co-activator p300 in mouse embryonic day-(E)-11.5 heart tissue, identified

over 3,000 heart enhancers and showed that those sites were surprisingly less deeply conserved in vertebrates [86]. Transgenic mouse assays of 130 candidate regions revealed many of them as functional. He et al. suggested that active subsets of p300 binding sites are binding sites for multiple co-localizing cardiac transcription factors, namely, GATA-binding protein 4, NK2 transcription factor-related, locus 5, T-box 5, SRF, and myocyte-enhancer factor 2A [87]. Asp et al. mapped epigenetic marks during myogenesis of mouse C2C12 cells. They identified novel regulatory elements flanking the myogenin gene and also investigated roles of the Polycomb repressive complexes in regulation of H3K27 methylation [88]. Glucocorticoids are known to elicit insulin resistance in skeletal muscle. Kuo et al. conducted ChIP-seq for glucocorticoid receptor (GR) and identified 173 GR target genes in mouse C2C12 myotubes. Among those, they demonstrated that p85α plays a critical role in glucocorticoid-induced insulin resistance and muscle atrophy [89].

3. Liver

Li S et al. described a genome-wide co-activation assay to globally identify transcription factors and cofactors in the PGC-1 pathway in mouse liver and identified BAF60a as a molecular link between the SWI/SNF chromatin remodeling complex and hepatic lipid metabolism [90]. HDAC3 is a member of class I HDACs. Feng et al. investigated genome localization of HDAC3 by ChIP-seq in mouse liver and found that binding of HDAC3 to the genome displays a circadian rhythm [91]. Genomic recruitment of HDAC3 in the liver is mediated by the circadian core factor Rev-erbα: loss of either HDAC3 or Rev-erbα resulted in hepatic steatosis; implicating rhythmic regulation of hepatic lipogenic enzymes is orchestrated by HDAC3-mediated epigenomics remodeling and is important for proper lipid metabolism in the liver [91]. In a subsequent study, the same group reported the unexpected finding that liver-specific depletion of HDAC3 resulted in higher insulin sensitivity despite severe hepatosteatosis [92]. They proposed rerouting metabolic precursors towards lipid synthesis and storage within lipid droplets and away from hepatic glucose production partly by induction of Perilipin 2.

4. Pancreatic Islets

Several groups have made effort to generate a genome-wide epigenomic map of human pancreatic islets. Gaulton et al. and Stitzel et al. respectively used FAIRE-seq and DNase-seq to map open chromatin genomic regions [93•, 94•]. It is noteworthy that they not only located regulatory elements in the islets, but also specified putative causal single nucleotide polymorphisms (SNPs) in genomic loci identified in genome-wide association studies (GWASs) of type 2 diabetes using the open chromatin maps as guidance [93•, 94•]. The use of genome-wide open chromatin maps as a reference for the determination of causal SNPs is particularly important because identifying causal SNPs has been one of the largest hurdles in GWASs. Bhandare et al. also conducted comprehensive epigenomic analyses in human pancreatic islets and showed that a substantial number of diabetes-associated non-coding SNPs are located in H3K4me1-enriched enhancer-like regions [95]. The liver and pancreas share a common origin. Hoffman et al. conducted ChIP-seq for FOXA2, HNF4A PDX1, and histone modifications in the mouse liver and pancreatic islets, and showed that both tissue-specific transcription factor binding and nucleosome remodeling complex recruitment are involved in determining tissue-specific gene expression [96].

5. Vascular and Other Systems

Pirola et al. exposed human aortic endothelial cells to high-glucose or low-glucose conditions for 2 days, generated histone H3K9/K14 acetylation and DNA CpG methylation maps, and identified unique hyperacetylation and CpG methylation signatures with proximal and distal patterns of regionalization associative with gene expression [97]. Miao et al. exposed a human THP-1 monocyte cell line to normal glucose or high glucose conditions, profiled the epigenetic change including H3K4me2 and H3K9me2 by using ChIP-on-chip, and identified candidate genes differentially regulated, a phenomenon also seen in monocytes of diabetic patients [98]. The same group took the same approach to analyze lymphocytes from patients with type 1 diabetes, identifying candidate genes that were epigenetically regulated in type 1 diabetes [99].

Epigenetic Regulation in Diabetes

Diabetic stimuli can trigger epigenetic alteration in the chromatin structure, which could serve as a mechanism of long lasting metabolic memory. Furthermore, direct evidence proving the role of chromatin-modifying enzymes in the development of metabolic diseases came from a series of studies using knockout mice. Here, we summarize investigations of epigenetic regulation in diabetes with emphasis on knockout mouse studies of chromatin-modifying enzymes.

1. Adipose Tissue

We reported that heterozygous knockout mice for cAMP response-element binding protein-binding protein (CBP histone acetyltransferase or KAT3A) exhibited increased insulin sensitivity despite lipodystrophy [100]. Detailed analyses revealed that this phenotype was caused by moderate inhibition of PPARγ, of which CBP is a co-activator, and increased serum adiponectin levels. More recently, 2 groups independently reported adult onset obese phenotype of *Jhdm2a* (KDM3A) knockout mice [101••, 102••]. JHDM2A

is a histone demethylase that catalyzes removal of H3K9 mono- and dimethylation. Tateishi et al. reported *Jhdm2a* knockout mice showed reduced expression of PPARα and uncoupling protein 1 (UCP1) in brown adipose tissue, leading to reduced β-adrenergic-stimulated glycerol release and oxygen consumption, and decreased fat oxidation and glycerol release in skeletal muscles [102]. Inagaki et al. reported that disruption of *Jhdm2a* epigenetically down-regulates genes involved in energy expenditure and fat storage in adipose tissue, leading to reduced whole-body energy expenditure [101••]. Hino et al. reported that loss of FAD-dependent lysine-specific demethylase-1 (LSD1), a demethylases for H3K4me1/2 and H3K9me1/2 (or KDM1), in mouse 3T3-L1 adipocytes caused increased expression of genes involved in energy expenditure and mitochondrial metabolism such as PGC1α [103]. They found that expression of LSD1 and its target genes was increased in obese adipose tissue, which was reversed by adenoviral transfer of shRNA for LSD1 in adipose tissue. They suggested that cellular FAD levels control LSD1-mediated regulation of these genes in 3T3-L1. It would be intriguing to determine whether this pathway works as a bona-fide nutrient sensor in a pathophysiological situation. Musri et al. demonstrated that knockdown of either LSD1 or H3K9 methyltransferase SETDB1 (or KMT1E) in mouse 3T3-L1 adipocytes resulted in markedly decreased and increased adipogenesis, respectively [104]. Altered H3K4 and H3K9 dimethylation at the promoter of the C/EBPα gene was responsible for the phenotype. Wang et al. isolated primary pre-adipocytes from conditional H3K27 methyltransferase *Ezh2(KMT6)*$^{flox/flox}$ knockout mice and showed that cells infected with adenovirus expressing Cre recombinase have a severe defect in adipogenesis [105]. They demonstrated that deletion of Ezh2 de-represses expression of anti-adipogenic Wnt, Pref1 and GATA3. Kamei et al. reported that expression of Dnmt3a is increased in obese adipose tissue [106]. *Dnmt3a* transgenic mice in adipose tissue exhibited higher expression levels of inflammatory cytokines.

In addition to chromatin-modifying enzymes, many factors involved in chromatin and transcription regulatory complexes were shown to be linked to adipocyte biology. Cho et al. demonstrated murine embryonic fibroblasts from homozygous conditional knockout mice for PTIP, a protein associated with H3K4 methyltransferases, leads to marked decreases of PPARγ expression and adipogenesis [107]. PTIP *(Paxip1)*$^{flox/-}$*; aP2-Cre* mice showed reduced amounts of brown adipose tissue and were prone to hypothermia when exposed to cold [107]. Examples of knockout mice of other co-regulators of chromatin/transcription exhibiting phenotype related to type 2 diabetes include: RIP140 (*Nrip1*) −/− mice showing dramatic activation of uncoupling protein 1 in white adipose tissue and reduced adiposity [108]; regulation of systemic energy balance by TIF2, SRC-1 and p/CIP [109, 110]; adipose-specific co-repressor *Ncor1* −/− mice showing insulin sensitivity resulting from reduced CKD5-mediated PPARγ ser-273 phosphorylation [111]; and mutant knock-in mice for SMRT (*Ncor2*)^{mRID1} showing increased adiposity and age-dependent metabolic defects [112, 113]. PGC1α is a co-activator that has multiple critical functions in metabolism of adipocytes, muscle, liver, and other organs and has been extensively reviewed by Handschin and Spiegelman [114].

2. Pancreatic Islets

Xu et al. employed a low-cell number ChIP protocol and demonstrated that the histone acetyltransferase P300 (KAT3B) and the histone H3 lysine 27 methyltransferase Ezh2 (KMT6) controls a cell fate of mouse embryonic endoderm cells into either liver or pancreas by pre-patterning chromatin states [115]. The H3K27 methyltransferase Ezh2 is a member of the PRC2 Polycomb complex and its role in the *Ink4a/Arf* locus that controls cellular senescence has been documented. Chen et al. reported epigenetic regulation of mouse pancreatic β-cell proliferation by Ezh2 (KMT6) [116•]. They showed that Ezh2 represses the *Ink4a/Arf* locus in β cells and that Ezh2 levels decline during aging of mice. Conditional deletion of *Ezh2* in β cells resulted in reduced H3K27 trimethylation at the *Ink4a/Arf* locus, leading to reduced β-cell mass, and mild diabetes. The phenotype was rescued by germ line disruption of *Ink4a/Arf.* Dhawan et al. also demonstrated that decline of levels of Bmi-1, a member of the PRC1 Polycomb complex, because of aging plays a critical role in the regulation of the *Ink4a/Arf* locus and the proliferating capacity of β cells and glucose homeostasis in mice [117•]. Chen et al. demonstrated platelet-derived growth factor receptor signaling controls age-dependent β-cell proliferation in an Ezh2-dependent manner in mice [118]. The homeodomain transcription factor Nkx2.2 is critical in cell-fate decisions in the pancreatic islet. Papizan et al. demonstrated that Nkx2.2 is a part of a large repression complex in mouse pancreatic β cells that includes DNMT3a, Grg3, and HDAC1 [119]. Nkx2.2 recruits the complex to the methylated Aristaless homeobox gene (Arx) promoter in β cells. The Nkx2.2 TN mutation abolishes the interaction of Nkx2.2 and Grg3 and results in ectopic expression of Arx in β cells, causing β-to-α-cell transdifferentiation. A corresponding β-cell-specific deletion of *Dnmt3a* is also sufficient to cause Arx-dependent β-to-α-cell reprogramming.

3. Skeletal Muscle

Barrès et al., using whole genome promoter-methylation analysis of skeletal muscle from normal glucose-tolerant and type 2 diabetic subjects, identified cytosine hypermethylation of the promoter of the PGC-1α gene in subjects with type 2 diabetes. They found that the highest proportion of cytosine methylation within PGC-1a was found within non-CpG

nucleotides and it was acutely increased by exposure to TNF-α or free fatty acids [120]. Barrès et al. examined DNA methylation status in skeletal muscle biopsy samples from human subjects after acute exercise [20••], which induced a dose-dependent expression of PGC-1α, PDK4, and PPAR-δ. Surprisingly, this was accompanied by a marked hypomethylation on each respective promoter, suggesting that the patterns of DNA methylation in differentiated non-dividing somatic cells are subject to more dynamic change than previously understood. Ling et al. examined DNA methylation status and expression of PGC-1α gene in human pancreatic islets from 48 non-diabetic and 12 type 2 diabetic multi-organ donors and found a two-fold increase in DNA methylation and reduced expression in diabetic donors [121]. Duteil et al. showed that skeletal muscle-specific ablation of TIF2 (*Ncoa2*) in mice resulted in increased mitochondrial uncoupling and protection from obesity and type 2 diabetes [122].

4. Liver

Zhong et al. demonstrated by using knockout mice that a NAD^+-dependent deacetylase SIRT6 functions as a histone H3K9 deacetylase and controls the expression of multiple glycolytic genes working as a co-repressor of HIF-1α [123]. Pan et al. performed an RNA interference screen and found that a histone H3 lysine 36 demethylase JHDM1a (KDM2A) was a key negative regulator of gluconeogenic gene expression [124]. Winkler et al. found that *Hdac6*-deficient mice exhibited attenuated gluconeogenic gene expression in response to dexamethasone treatment by modulation of glucocorticoid receptor translocation [125]. Ehara et al. investigated DNA methylation in the promoter region of Glycerol-3-phosphate acyltransferase 1 in mouse liver and showed that it is methylated in neonates but demethylated in adult mice, accompanied by active chromatin conformation, recruitment of SREBP1c, and activation of the gene expression, all of which coincide with change in nutrition and necessity for hepatic lipogenesis around the weaning period [126]. Interestingly, maternal overnutrition resulted in early demethylation and increased gene expression in pups. It would be intriguing to determine if this difference persists until adulthood because that may explain metabolic memory.

5. Vascular and Other Systems

Results from studies examining involvement of epigenetic regulation in the vascular system especially highlighted the role of epigenetics in long-lasting metabolic memory. Es-Osta et al. reported that transient hyperglycemia induces activating epigenetic change in the promoter of the NFκB p65 subunit in human and murine aortic endothelial cells. Interestingly, this epigenetic change persisted six days after glucose was normalized [127]. Similarly, Villeneuve et al. found H3K9me3 levels significantly decreased at the promoters of key inflammatory genes in vascular smooth muscle cells of *db/db* mice due to reduced levels of H3K9 methyltransferase Suv39v1 (KMT1A) [128]. The histone H3 lysine-27 demethylase Jmjd3 (KDM6B) is induced in response to lipopolysaccharide and contributes to the control of as much as 70 % of LPS-induced genes [26, 129]. Recently, the JMJD3-IRF4 axis has been shown to regulate M2 macrophage polarization in mice [130]. Epigenetic mechanisms in the vascular system are thoroughly reviewed by Reddy et al. [131].

Epigenome-Modifying Enzymes as a Class of Druggable Target

Since epigenetic gene regulation has been gradually recognized as a dynamic and reversible process, enzymes catalyzing epigenetic modification are emerging as druggable classes of enzymes [11]. Currently, inhibitors of HDAC (Vorinostat and Romidepsin) are approved for clinical use in treatment of cutaneous T-cell lymphoma [11]. Many other HDAC inhibitors are in clinical trials mainly for cancer treatment. A GlaxoSmithKline group recently described the high-resolution crystal structure of mouse and human JMJD3 and reported the first small-molecule catalytic site inhibitor that is selective for theH3K27me3-specific JMJ subfamily [132]. Considering the role of epigenetics as a link between environmental stimuli and development of type 2 diabetes and the metabolic phenotypes of knockout animals, including those of *CBP (KAT3A)* [100], *Jhdm2a (KDM43A)* [101••, 102••], *Lsd1 (KDM1)* [103], *Ezh2 (KMT6)* [105, 116], and *Sirt6* [123, 125], development of drugs modulating epigenetic modification for treating type 2 diabetes and related disorders is expected. For such drugs, though, a clear benefit with an acceptable safety and tolerability is necessary for treatment of chronic diseases such as diabetes; this is one of the big challenges that must be met especially because of the fundamental role of these enzymes as general factors in the regulation of global gene expression patterns [11].

Conclusions

We have provided an overview of the latest findings and the state of knowledge about epigenetics, epigenomics and their roles in type 1 and type 2 diabetes. The discovery of a number of chromatin-modifying enzymes and the development of high-throughput sequencing technology has boosted our understanding of epigenetic regulation in the past decades. Although the challenge to fully understand the role of epigenetic regulation in diabetes research continues, such effort will likely elucidate the fundamental mechanisms underlying the etiology of type 1 and type 2 diabetes

and their complications and facilitate the development of rational approaches to the treatment of diabetes.

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白色脂肪細胞のエピジェネティックス

Epigenetics of white adipocytes

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◎肥満症，メタボリックシンドローム，糖尿病の急増は社会的な問題である．白色脂肪細胞は余剰なエネルギーの貯蔵臓器として，アディポカインを分泌する内分泌細胞として全身のエネルギー代謝において重要な役割を果たしている．近年のゲノムサイエンス分野の革新的な技術である次世代シークエンサーにより転写因子やエピゲノムの網羅的な解析が可能となり，既存の方法ではわからなかった新しい視点から脂肪細胞の転写調節機構が明らかにされつつある．本稿では，著者らが最近行った脂肪細胞特異的なオープンクロマチン領域解析(FAIRE-seq)を含めて最新の脂肪細胞のエピジェネティックス研究をレビューする．

Key word：脂肪細胞分化，エピゲノム，オープンクロマチン，PPARγ，次世代シークエンサー

真核生物のゲノムDNAは，ヒストンと結合したヌクレオソームを基本構造として核内に複雑に折りたたまれている(クロマチン構造，図1)．遺伝子が発現するためにはクロマチン構造がダイナミックに変化する必要があり，その制御にはヒストンやDNAなどの翻訳後修飾が重要な役割を果たすことが明らかにされてきた．エピジェネティックスという言葉は1940年代にConrad Waddingtonが，幹細胞が各種細胞に系統分化していく発生過程を念頭においてはじめて用いた[1)]．現在では，「DNA配列を伴わない細胞の表現型の変化を生みだすさまざまなDNAやヒストンの修飾を含む分子機構」を示し，その総体としてエピゲノムという用語が使用される．近年開発された次世代シークエンサーにより転写因子やエピゲノムの網羅的な解析が可能となり，既存の方法ではわからなかった新しい視点から脂肪細胞の転写調節機構が明らかにされつつある．

わが国を含め全世界で肥満症，メタボリックシンドローム，糖尿病が急増している．その病態において，脂肪細胞の機能の異常は重要な役割を果たすと考えられる．脂肪細胞は余剰エネルギーの貯蔵のみならず，アディポカインを分泌し全身の代謝を制御・調節する分子を積極的に分泌する内分泌臓器として役割をもつ[2)]．インスリン抵抗性改善薬チアゾリジン誘導体は脂肪細胞分化のマスターレギュレーターperoxisome proliferator-activated receptor γ(PPARγ)のアゴニストであり，脂肪細胞における転写制御が全身の糖脂質代謝の鍵となることが明らかにされてきた．本稿では，近年進んできた白色脂肪細胞におけるエピゲノム解析について概説する．

次世代シークエンサーによる白色脂肪細胞のエピゲノム解析

近年のゲノムサイエンス分野の革新的な技術である次世代シークエンサーにより，転写因子やエピゲノムの網羅的な解析が可能となった．特異抗体を用いたクロマチン免疫沈降(chromatin immunoprecipitation：ChIP)と次世代シークエン

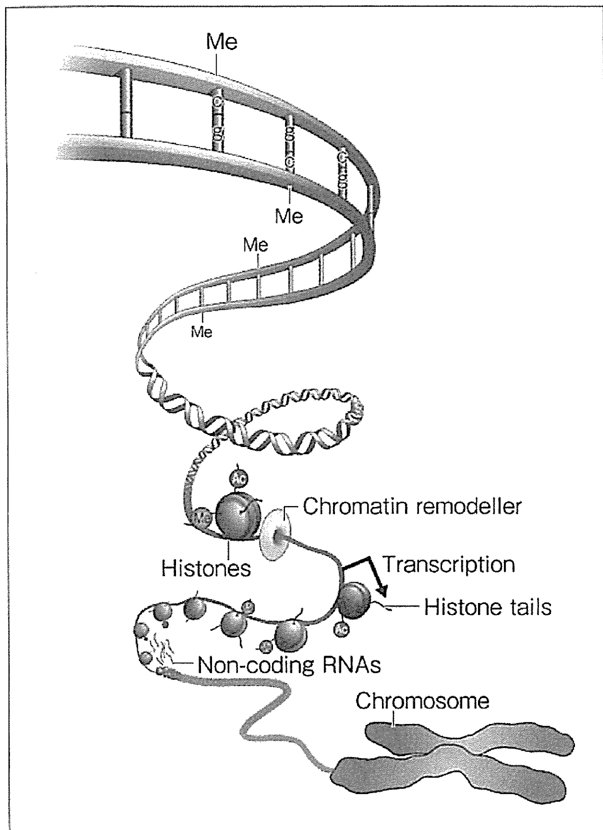

図 1　クロマチン構造とDNA・ヒストン修飾
(文献[20]より改変)

サーを組み合わせることにより(ChIP-seq)，転写因子の結合領域および特定のヒストン修飾などのゲノムワイド解析が可能となる．また，ゲノム上の転写制御領域はオープンクロマチン構造をとることが知られているが，そのような領域を同定する方法として，古典的なDNase I hypersensitivity アッセイやFormaldehyde-Assisted Isolation of Regulatory Elements(FAIRE)と次世代シークエンサーとを組み合わせることにより(DHS-seq とFAIRE-seq)，全ゲノムレベルで転写制御領域を同定することが可能になった．

脂肪細胞分化ではこれまで，PPARγやC/EBPなどの転写因子のカスケードが重要な役割を果たすことが知られていた．脂肪細胞分化におけるPPARγの結合領域であるChIP-on-chipや，ChIP-seqによるゲノムワイド解析が報告された[3,4]．これらのゲノムワイド解析による新しい知見のひとつは，PPARγとC/EBPαがゲノム上の多くで，同じ領域に共存(co-localization)しており，たがいに協調して標的遺伝子を調節していることがあげられる．分化刺激に伴う早期のエピゲノム変化の解析から，早期に誘導されるC/EBPβとグルココルチコイド受容体が一過性にゲノムの特定の領域に結合して一種のエピジェネティックな移行状態を形成することにより，分化後期のPPARγとC/EBPαによる脂肪細胞分化のプログラムが進むことができる機構を示した[5]．

Siersbæk らは，分化早期に誘導される転写因子のChIP-seqやDHS-seqによるオープンクロマチンの検討により，分化誘導剤添加の4時間後を中心にダイナミックなクロマチン構造の変化が起こり，そこに数種類の早期に誘導される転写因子が同時に結合する“hot spot”とよばれる領域を形成していることを示した[6]．なかでもC/EBPβは同領域の他の転写因子の結合に必須の役割を果たすことを示した．

異なる転写因子が同領域に共存する“co-localization”という概念は，ゲノムワイド解析によりはじめて明らかにされてきたもののひとつである．脂肪細胞においてPPARγはC/EBPαと共存しているが，マクロファージにおいてPPARγはとくに免疫系統の遺伝子の近傍で，血球分化に重要な役割を果たすPU.1と特異的に共存していた[7]．同じ転写因子が異なる細胞において異なる標的遺伝子をもち，異なる生理作用をもつ現象の原因として，共存においてパートナーとなる転写因子の違いによる可能性が示唆される．

江口らは，qPCRをベースとしたDNase I hypersensitivity アッセイを脂肪細胞分化に特異的な27個の遺伝子の近傍において施行して得られた脂肪細胞特異的な転写制御領域のDNA配列のモチーフ解析により，IRF転写因子が脂肪細胞分化の新しい制御因子であることを示した[8]．

Mikkelsen らは，マウスの白色脂肪細胞とヒト脂肪細胞においてPPARγ結合領域をChIP-seqで網羅的に行った．PPARγの標的遺伝子においては多数のPPARγの結合領域が遺伝子周囲に存在しているが，これまでの予測を覆して個々のPPARγの結合領域は種間であまり保存されていないことを示した[9]．これらの違いはトランスポゾンの挿入によるPPARγ結合配列の変化に伴っていた．彼らはさらに，脂肪細胞特異的なヒストンアセチル化を指標に得られた領域のDNA配列

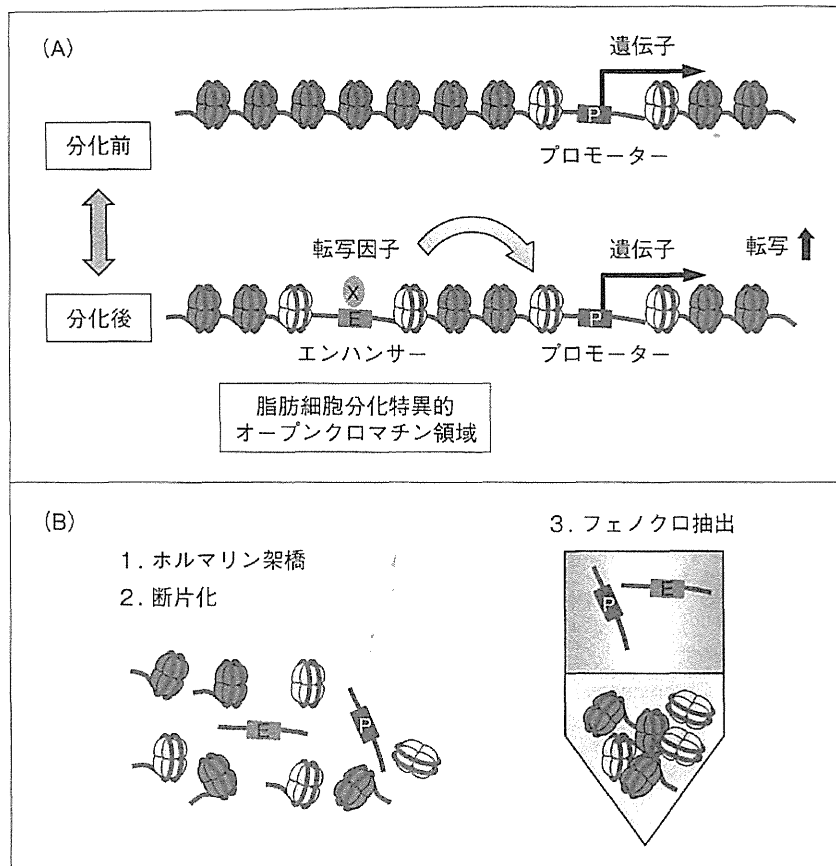

図 2　FAIREによるオープンクロマチン領域の検出
A：エンハンサーやプロモーターなどの制御領域はオープンクロマチン構造をとる．分化前後で変化するオープンクロマチン領域には分化を制御するエンハンサー領域が含まれる．
B：FAIRE の原理．

のモチーフ解析により，PLZF と SPF という新しい転写制御因子を同定した．

著者らは FAIRE-seq を用いることにより，脂肪細胞に特異的なオープンクロマチン領域をゲノム上にマッピングした[10]．FAIRE は細胞でホルマリン架橋を行った後にソニケーションを行ってフェノールクロロホルム抽出することによりヌクレオソームがとれた DNA を選択的に抽出する方法である(図 2)[11]．脂肪細胞で 30,000 カ所以上のオープンクロマチン領域を同定したが，約 7 割を占める非プロモーター領域のオープンクロマチン領域はエンハンサーに特徴的とされるヒストン修飾 H3K4me1^{+}/me3^{-}で特徴づけられ，これらの領域が遠位エンハンサーであることが示唆された．非プロモーター・オープンクロマチン領域はプロモーター領域と比較して細胞の分化によりダイナミックに変化しており，KLF15，C/EBPα や AdipoR2 など，これまでの近位プロモーターを用いたレポーターアッセイでは同定できなかった脂肪細胞特異的な転写制御領域がイントロンなどの遠位に複数存在することが明らかとなった(図3)．

脂肪細胞分化のマイクロアレイの解析では，一遺伝子に存在するこのようなエンハンサーの“数”が適切な転写制御に重要な因子であることが示唆された．さらに，これらの領域の DNA のモチーフ解析により，既知の PPARγ，C/EBP，Zfp423 に加えて NFI 転写因子ファミリーの結合モチーフが濃縮されており(図 4)，過剰発現やノックダウンの解析(図 5)から NFI が脂肪細胞分化を制御する新しい因子であることを見出した[10]．Wnt 経路は脂肪細胞分化の重要な抑制経路であるが，β カテニンの ChIP-on-chip 解析により Okamura らは核内受容体 COUP-TF II が重要な役割を果たすことを示した[12]．若林らは PPARγ の ChIP-

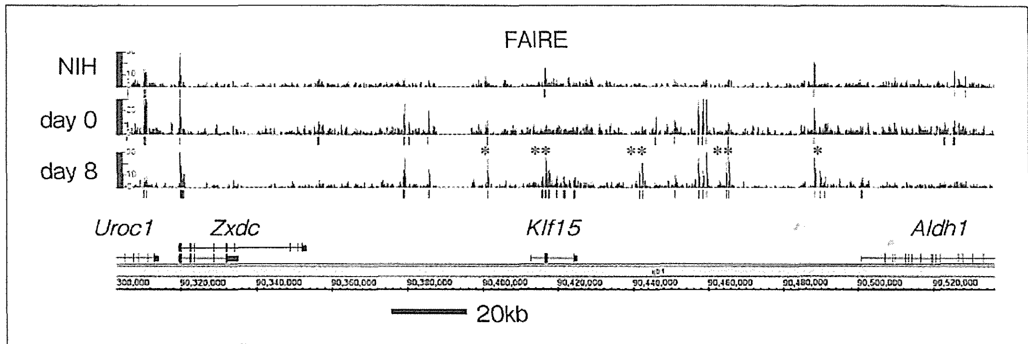

図 3 *Klf15*遺伝子領域のFAIREシグナル[10]
上段から NIH-3T3, 3T3-L1 day 0, 3T3-L1 day 8. 下線は有意なピーク(FDR＜10^{-4}).
*：脂肪細胞特異的なオープンクロマチン領域.

Motif	Name	Corrected *p*-value	Enrichment ratio (Ad/pAd)	Logo
M00193	NF-1	7.9E−27	1.60	
M01196	CTF1	5.1E−22	1.55	
M01100	LRF	2.6E−20	1.65	
M00528	PPAR	2.7E−12	2.14	
M01728	EAR2	1.2E−09	1.47	
M01031	HNF4 (PPAR)	3.8E−08	2.06	
M01772	C/EBP	1.7E−07	2.69	
M00109	C/EBPβ	3.1E−07	1.51	

図 4 脂肪細胞特異的オープンクロマチン領域に濃縮される転写因子の結合モチーフ解析
(TransfacデータベースRelease 2010.4)[10]
PPAR や C/EBP のほかに NFI(CTF1)のモチーフが濃縮している.

on-chip 解析により，ヒストン H4K20 のモノメチル化酵素 PR-Set7/Setd8(KMT5A)が PPARγ の標的遺伝子であり，PPARγ による標的遺伝子の転写制御メカニズムに重要であることを示した[13].

白色脂肪細胞における エピジェネティック制御

エピジェネティックな遺伝子の転写制御が直接，肥満や糖尿病の発症にかかわることは，ヒストン修飾酵素のノックアウトマウスの表現型から

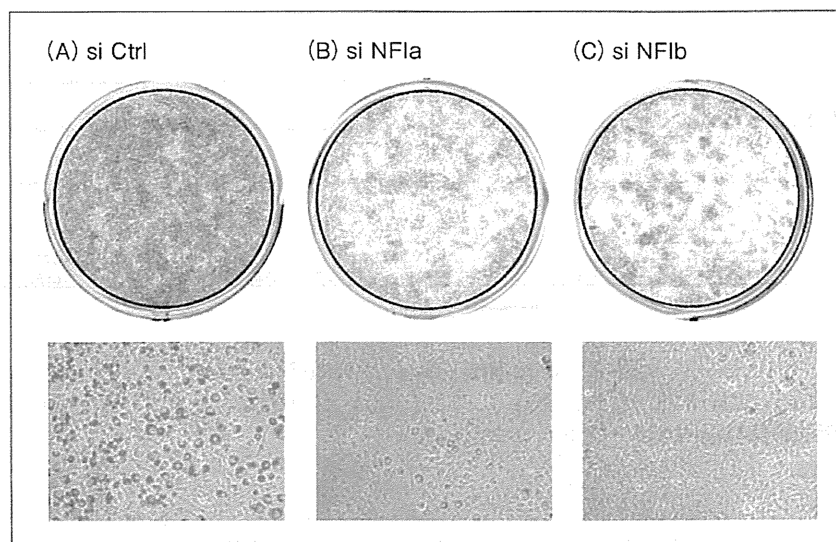

図 5　3T3-L1脂肪細胞分化におけるNFIA，NFIBのノックダウンの効果[10)]

示される．ヒストンアセチル化酵素であるCBPのヘテロ欠損マウスは，著しい脂肪委縮を示すにもかかわらず良好なインスリン感受性を示した[14)]．この表現型にはPPARγ活性の中等度の抑制と血清アディポネクチンの上昇が重要であることが示唆された．

一方，近年ヒストンH3K9の脱メチル化酵素であるJhdm2aの欠損マウスが肥満の表現型を示すことが2つの独立したグループから報告された[15,16)]．立石らは，Jhdm2a欠損マウスが褐色脂肪組織においてPPARα，UCP1発現，脂質分解と脂肪酸のβ酸化が低下および酸素消費量が減少していることを示した[16)]．稲垣らは，脂肪組織におけるエネルギー消費にかかわる遺伝子の発現が減少するために全身のエネルギー消費が減少することをメカニズムとして示した[15)]．最近，日野らは脂肪細胞におけるヒストンH3K4とK9の脱メチル化酵素であるLSD1の欠損の表現型から，LSD1がエネルギー消費やミトコンドリア関連遺伝子を標的遺伝子としており，興味深いことに肥満脂肪組織においてLSD1の発現が増加していること，shRNAアデノウイルスによりその発現を制御することでミトコンドリア関連遺伝子の発現が回復することを示した[17)]．LSD1はFAD依存性の脱メチル化酵素であることから，栄養環境が直接エピゲノムを制御する可能性については興味深い．Wangらはヒストン H3K27 メチル化酵素のEzh2のコンディショナルノックアウトマウスを用いて，Ezh2が脂肪細胞分化の抑制因子であるWntの制御を介して脂肪細胞分化を制御することを示した[18)]．同グループは，ヒストンH3K4メチル化酵素に結合するコファクターであるPTIPのコンディショナルノックアウト細胞ではPPARγが強く抑制されて脂肪細胞分化に障害があり，aP2-Creによるノックアウトマウスは褐色脂肪細胞の分化が抑制され，寒冷刺激に弱いことを示した[19)]．

おわりに

最近の白色脂肪細胞におけるエピジェネティックス研究について，次世代シークエンサーを含めたゲノムワイド解析とヒストン修飾酵素による脂肪細胞や肥満の制御について概説した．本稿では触れなかったが，エピゲノムは環境因子とゲノムの接点として，とくに糖尿病の分野では，オランダ飢饉時に胎児であったヒトが成人で糖尿病などの発症リスクが高くなること，また，UKPDSなどの大規模臨床試験で血糖コントロールの糖尿病合併症発症への影響が血糖正常化後も長期にわたって“記憶”されている現象(遺産効果)への関与が示唆されている．今後，脂肪細胞分化のみならず，広くメタボリックシンドロームに関連した病態生理において，ゲノムワイド解析が既存の研究手法とはまったく異なるあらたな知見を与えてくれるものと期待される．