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MULTI-AUTHOR REVIEW

Developmental and environmental epigenetic programming of the endocrine pancreas: consequences for type 2 diabetes

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Abstract The development of the endocrine pancreas is controlled by a hierarchical network of transcriptional regulators. It is increasingly evident that this requires a tightly interconnected epigenetic "programme" to drive endocrine cell differentiation and maintain islet function. Epigenetic regulators such as DNA and histone-modifying enzymes are now known to contribute to determination of pancreatic cell lineage, maintenance of cellular differentiation states, and normal functioning of adult pancreatic endocrine cells. Persistent effects of an early suboptimal environment, known to increase risk of type 2 diabetes in later life, can alter the epigenetic control of transcriptional master regulators, such as Hnf4a and Pdx1. Recent genome-wide analyses also suggest that an altered epigenetic landscape is associated with the β cell failure observed in type 2 diabetes and aging. At the cellular level, epigenetic mechanisms may provide a mechanistic link between energy metabolism and stable patterns of gene expression. Key energy metabolites influence the activity of epigenetic regulators, which in turn alter transcription to maintain cellular homeostasis. The challenge is now to understand the detailed molecular mechanisms that underlie these diverse roles of epigenetics, and the extent to which they contribute to the pathogenesis of type 2 diabetes. In-depth understanding of the developmental and environmental epigenetic programming of the endocrine pancreas has the potential to lead to novel therapeutic approaches in diabetes.

Keywords Epigenetics · Islets · Pancreas · Diabetes · Development · Environment

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Introduction

Diabetes mellitus is a chronic metabolic disease of "epidemic" proportions; its global prevalence was estimated as 285 million adults (6.4 %) in 2010 and is expected to further rise to approximately 439 million (7.7 %) by 2030 [1]. Type 2 diabetes (T2D), sometimes termed non-insulin-dependent diabetes mellitus, is the predominant form and accounts for more than 90 % of all cases. The disease is characterized by insulin secretory dysfunction of the pancreatic β cells combined with insulin resistance [2], but the primary mechanisms are still debated. Current therapeutic regimens are often unable to stop the progression of the disease towards the development of microvascular and macrovascular complications [3], which together put a huge economic burden on public health care systems.

T2D is a multifactorial disorder influenced by interactions between multiple susceptibility genetic loci and the environment. Strong familial clustering is indicative of a major role for genetic factors, estimated to account for 30–70 % of the total risk for T2D [4]. Studies performed

over the past two decades led initially to the identification of genes responsible for rare, monogenic forms of diabetes such as maturity onset diabetes of the young (MODY), neonatal diabetes, mitochondrial diabetes, and lipoatrophic forms of diabetes [5]. Although mutations in these genes account for only 2–5 % of all cases of diabetes, their discovery provided novel insights into the pathways that regulate glucose homeostasis. Genome-wide association studies (GWAS) in T2D have also contributed significantly to our understanding of the genetics of this disease. So far, more than 40 loci have been identified that increase the risk for T2D [6]. Interestingly, most of the associated genes appear to be enriched in the β cell and to have the potential to regulate β cell mass and/or function [7]. Although the combined effects of these genetic variants only explain around 10 % of T2D heritability, they point towards a major role for pancreatic β cell dysfunction in the etiology of this disease.

The environment acting across the life-course plays an important role in T2D pathogenesis. Risk factors include reduced physical activity, obesity, low birth weight, and advancing age [8, 9]. Epigenetics has attracted particular attention in the last few years mainly because it plays a central role at the interface between the genome and the environment. It has been proposed that the environment increases the risk of T2D by affecting gene expression through epigenetic modifications [10, 11]. Epigenetics refers to "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states", caused by mechanisms other than changes in the underlying DNA sequence [12]. These structural adaptations are brought about by reversible epigenetic marks that modulate genome function. Epigenetic marks include DNA modifications (such as 5-methylcytosine [13], 5-hydroxymethylcytosine [14], 5-formylcytosine and 5-carboxylcytosine [15]), post-translational modifications of histones (methylation, acetylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, deimination, proline isomerization, *O*-GlcNAcylation [*O*-linked β-D-*N*-acetylglucosamine] and tail clipping) [16], histone variants [17], and alternative nucleosome positioning [18]. These modifications are laid on the chromatin by a variety of chromatin and DNA-binding proteins with enzymatic activities [19–21], as well as non-coding RNAs [22], all of which act as epigenetic initiators that translate environmental cues (epigenators) into specific epigenetic states [23]. Importantly, epigenetic systems must ensure both stability and flexibility of chromatin states [24]. The regulation of transcription requires the remodeling of chromatin via DNA and histone modifications, the alteration of nucleosomal distribution and the adjustment of higher-order chromatin structure. A key question is how epigenetic systems can show at the same time robust epigenetic memory that is central to maintenance of cell differentiation states, and flexibility to adapt and respond to various environmental challenges. The response to an initiating signal may result in chromatin events that are transient, with a return to "basal" levels as the signal fades, whereas others persist long term and are even transmitted through generations. A full understanding of how short-term signals can induce long-term heritable changes in gene expression will provide new insights on how growth and developmental adaptations influence postnatal phenotypes, leading to increased risk of disease and the onset of age-related diseases like type 2 diabetes [10, 25].

In this review, we discuss recent advances in the epigenetics of the endocrine pancreas, from roles in cell lineage specification and maintenance of differentiated states, to the transduction of environmental signals that impact genome function. We start by summarizing the evidence in support of key roles for epigenetic mechanisms in the development and function of the endocrine pancreas. We then discuss examples of environmental challenges that induce epigenetic changes in the endocrine pancreas in early life and during aging. Finally, we review evidence that links abnormal epigenetic marking of genes to the development of diabetes, with a focus on T2D, and discuss the molecular mechanisms that may underpin environmentally induced epigenetic metabolic programming.

The epigenetic control of endocrine pancreatic development and function

Overview of the pancreatic developmental program

The pancreas consists of several distinct cell populations arising from multipotent endodermal cells that will give rise to ductal, exocrine, and endocrine cells [26]. The exocrine compartment accounts for more than 95 % of the pancreatic tissue in adult animals and is composed of acinar cells, which synthesize digestive enzymes, and ductal cells that line the channels that transport these secretions into the gastrointestinal tract. The endocrine compartment is comprised of five different hormone-secreting cell types (glucagon-secreting α cells, insulin-secreting β cells, somatostatin-releasing δ cells, ghrelin-producing ε cells, and pancreatic polypeptide-secreting PP cells) that aggregate to form the islets of Langerhans [26].

The pancreas develops from the embryonic foregut and is therefore of endodermal origin [26–29]. In mouse, pancreatic development begins around embryonic day E8.5 with the formation of two pancreatic primordial buds (dorsal and ventral), which contain multipotent progenitor cells that express the transcription factor Pdx1 (pancreatic and duodenal homeobox 1). Pdx1 also plays important roles later in development, being required for prenatal pancreas morphology, postnatal maintenance of insulin production and

glucose-sensing, and control of the balance between endocrine and exocrine pancreatic components. Gut rotation at E11.5 leads to the fusion of the dorsal and the ventral buds, followed by their expansion into the surrounding mesenchyme. Between E12 and E14, the duct Pdx1^{+} progenitor cells located in a central position of the developing pancreas and in close apposition to the vasculature, follow an endocrine specification path, which is initially marked by the expression of the transcription factor Ngn3 (neurogenin 3) [30]. Several transcription factors, which act downstream of Ngn3, regulate the formation of various cell types within the islets. Two of these transcription factors, Pax4 (paired box gene 4) and Arx (aristaless-related homeobox), play crucial and antagonistic roles in the distribution of the endocrine subtype lineages: Pax4 supports β/δ cell fate, while Arx promotes the development of α and PP cells. By E15.5, fate specification of all endocrine cell types is completed. The migration of the differentiated endocrine cells and the formation of the islets of Langerhans take place between E16.5 and birth. After birth, there are two waves of β cell neogenesis in rodents, one immediately after birth and the other 2–3 weeks after birth, which are separated by a transient period of increased apoptosis [31, 32]. Adult mouse islets comprise ~75 % β cells, ~20 % α cells, and ~5 % other endocrine cells, while adult human islets contain ~50 % β cells, ~40 % α cells, ~10 % δ cells and very few PP and ε cells [26–30].

Epigenetic mechanisms and cell-fate decisions during the development of the endocrine pancreas

Epigenetic mechanisms play important roles during the cell-fate decisions that mark the development of the pancreas (summarized in Fig. 1). Early pluripotent cells of the embryo develop into multipotent mesoderm, ectoderm, and endoderm germ cell layers. In pluripotent embryonic stem (ES) cells, the CpG-rich promoters (genomic regions with high frequency of CpG dinucleotides, also called "CpG islands") of a large fraction of genes are marked concomitantly with trimethylation of histone H3 lysine 4 (H3K4me3—histone mark enriched in differentiated cells at transcriptional start sites of genes activated by the Trithorax [TrxG] complex), and H3K27me3 (found in differentiated cells at genes transcriptionally repressed by Polycomb group [PcG] proteins), status known as bivalent [33]. It has been proposed that the function of bivalency in pluripotent cells is to silence developmental genes while keeping them "poised" for activation, thus constituting a chromatin-based mechanism for maintaining pluripotency. Lineage-specific transcriptional competence can be associated with a resolution of this bivalent state, with loss of either H3K27me3 or H3K4me3 and consequent activation or repression of lineage-specific genes, respectively [33, 34]. In the mouse, the epigenetic status of the *Pdx1* gene fits the above model: the bivalent state is still present in the ventral foregut endoderm cells, before

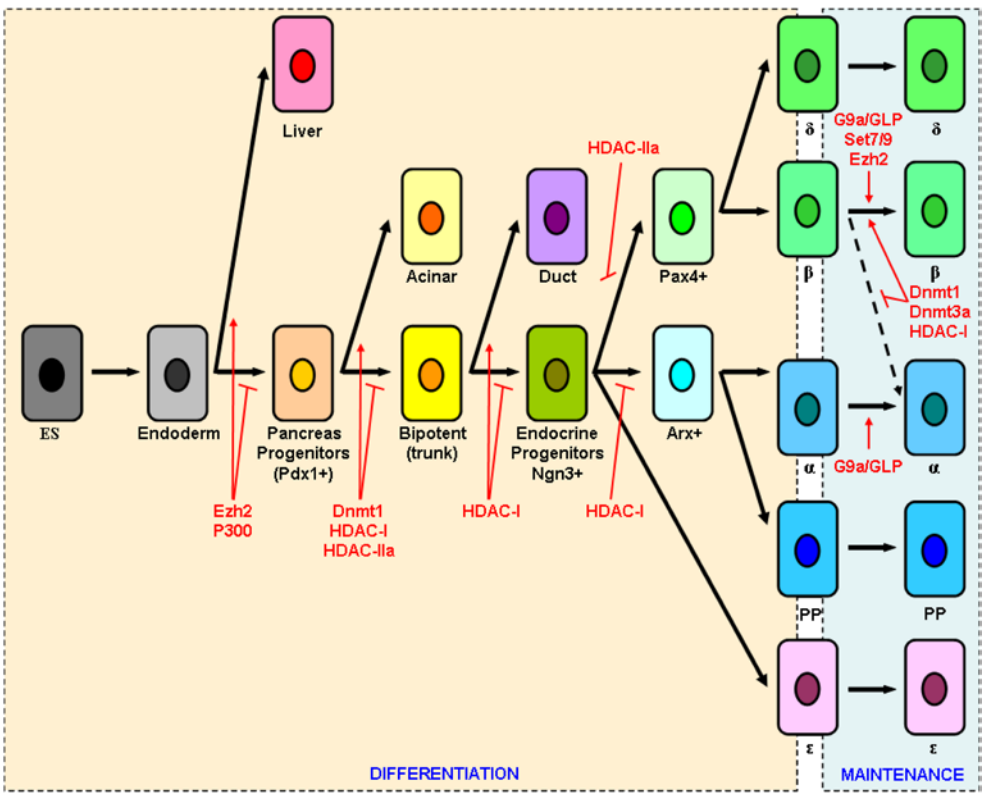


Fig. 1 The role of epigenetic mechanisms in cell lineage determination and maintenance of differentiated states of the endocrine pancreas. The *diagram* depicts the main steps in pancreas development and the role of several specific epigenetic regulators in these transitions (*red arrows* see text for details). *ES* embryonic stem cells

the appearance of the ventral pancreas progenitor cells [35], and H3K27me3 is then lost in E10.5 Pdx1$^+$ multipotent progenitor cells [36]. Loss of H3K27me3 is also observed in E10.5 Pdx1$^+$ progenitor cells at other loci with bivalent domains, including transcription factors that regulate the development of the nascent pancreas (e.g., *Foxa2*, *Gata4*, *Gata6*, and *Hnf1b*) [36]. However, many genes retain the bivalent chromatin status in E10.5 Pdx1$^+$ cells in order to suppress alternative developmental programs, as well as the premature expression of the genes that characterize terminally differentiated pancreatic cells [36].

The observation that a subset of genes marked by H3K27me3 in E10.5 Pdx1$^+$ progenitor cells are no longer repressed in adult β cells, has led to the proposal that the core β cell gene activity program remains largely suppressed by H3K27me3 in pancreatic progenitors and is selectively derepressed in the pancreatic endocrine lineage during later stages of differentiation [36]. A wave of de novo H3K27 tri-methylation also occurs in the β cell lineage after the pancreatic progenitor stage, affecting predominantly CpG-poor genes. These events are likely to be important for the repression of alternative cell fates (e.g., repression in pancreatic β cells of *Cpa2*, an acinar-specific gene that encodes carboxypeptidase A2) [36]. In the context of the complex roles played by H3K27me3 during pancreatic development, it is interesting to note that a specific deletion in the foregut endoderm of the histone methyltransferase Ezh2 (enhancer of zeste homolog 2—a component of the PcG complex PRC2, which mediates the addition of this epigenetic mark) leads to a marked expansion of Pdx1$^+$-positive cells in the ventral bud at E10, at the expense of liver bud development [35]. The increase in number of Pdx1$^+$-positive cells cannot be explained by cell proliferation, demonstrating that *Ezh2* is a modulator of the choice between liver and pancreatic programs [35]. Ezh2 is proposed to indirectly promote the liver program by restraining the extent of ventral pancreatic specification in the endoderm [35].

Acetylation and deacetylation of histones, which affect gene transcription via condensation–decondensation of chromatin, also play modulator roles in cell-fate decisions during pancreatic development. In E10 embryos with a heterozygous deletion of the histone acetyltransferase P300 (which acetylates lysine residues H3K9, H3K14, H2BK5, and H4K5), more Pdx1$^+$ cells are formed at the ventral pancreatic bud, while the liver bud is smaller [35]. Thus, P300 seems to promote the choice for liver over ventral pancreas fate. Histone deacetylases (HDACs) are also emerging as important modulators of the timing and determination of pancreatic cell fates. HDAC class I (HDAC1-3 and 8) seem to promote the endocrine lineage development at several key steps [37]. Indeed, treatments with HDAC-I inhibitors (such as valproic acid or MS275) in an in vitro model, in which endocrine and exocrine cells develop from E13.5 rat pancreata, lead to a dramatic decrease in acinar lineage differentiation, whereas ductal lineage differentiation is strongly enhanced. Moreover, the expression of the pro-endocrine transcription factor Ngn3 is strongly enhanced and maintained by the HDAC-I inhibitors, leading to an increased pool of Ngn3$^+$ endocrine progenitor cells [37]. Later during development, HDAC-I inhibit Arx activity, restricting the pool of α cells and PP cells [37]. In contrast, HDAC class IIa (HDAC4, 5, 7, and 9) limit the final steps in β and δ cells differentiation pathways by restricting the expression of Pax4 [38]. In *Hdac5*$^{-/-}$ and *Hdac9*$^{-/-}$ mice there is an increased number of β cells, while *Hdac4*$^{-/-}$ and *Hdac5*$^{-/-}$ mice have more δ cells [38]. Moreover, MC1568, a specific HDAC-IIa inhibitor activates *Pax4* expression in E13.5 rat pancreatic explants and increases the frequencies of β and δ cells [38].

The role of DNA methylation during early pancreas development is less understood. However, in mutant zebrafish with loss of DNA methyltransferase 1 (Dnmt1) catalytic activity, the pancreas forms normally but begins to degenerate 84 h post-fertilization through extensive apoptosis of the acinar cells, while the endocrine cells and the ducts are largely spared [39, 40]. Interestingly, these mutants also show an increased capacity for β cell regeneration in an inducible model of pancreatic β cell ablation, suggesting that DNA methylation plays important roles in the differentiation of pancreatic progenitors towards the β cell fate [40].

Epigenetic mechanisms and the long-term stability of differentiated cell states in the endocrine pancreas

As shown in the previous section, epigenetic mechanisms are important modulators of cell-fate decisions in the development of the endocrine pancreas (Fig. 1). Moreover, epigenetic mechanisms are also thought to be important players in the maintenance of lineage identity of somatic tissues, thus safeguarding from de-differentiation and reprogramming processes (also summarized in Fig. 1). Several recent studies based on genetic approaches (conditional deletions in mouse or siRNA knockdowns), pharmacologic approaches (small molecules targeting chromatin-modifying enzymes), epigenome mapping, and characterization of chromatin interactions have started to shed light on the role of epigenetic mechanisms in the maintenance of cellular identity of the endocrine cell during development and postnatal life. In the section that follows, we will give examples of the contribution of epigenetic mechanisms to long-term maintenance of identity of the endocrine cell.

Genetic and pharmacologic approaches

In mouse β cells, the promoter of the α cell fate regulator *Arx* is methylated and bound by the methyl-binding protein

MeCP2, which in turn recruits the histone methyltransferase PRMT6, an enzyme that methylates H3R2 (histone H3 arginine 2) [41]. The *Arx* promoter also binds a repressor complex comprised of the homeodomain transcription factors Nkx2.2 and Nkx6.1, the corepressor Grg3 (Groucho-3) and the de novo DNA methyltransferase Dnmt3a, which in turn recruits HDAC1 to the locus [42]. Together, these mechanisms lead to the transcriptional silencing of Arx in β cells. Deletion of the maintenance DNA methyltransferase Dnmt1 in differentiated β cells leads to gradual loss of DNA methylation and activation of the *Arx* gene and these events are associated with a conversion of β into α cells [41]. Interestingly, this cell identity change is only observed in older mice (over 8 months), even though the *Dnmt1* gene is deleted as early as β cells are specified during embryogenesis. This delay is thought to be explained, at least in part, by the amount of time needed for the slow rate of β cell proliferation to dilute the levels of DNA methylation established at the *Arx* locus prior to deletion [41]. However, there is also evidence that during the early stages of post-β cell specification, *Arx* repression involves active de novo DNA methylation at this locus, mediated by a large repression complex that includes Dnmt3a, Nkx2.2, Grg3, and HDAC1. Accordingly, deletion of *Dnmt3a* in β cells results in Arx-dependent β-to-α cell reprogramming as early as 3 months of age [42].

When the histone methyltransferase Ezh2 is deleted in mouse β cells, the level of the repressive mark H3K27me3 falls significantly at the *Cdkn2a* locus (which encodes two cell-cycle repressors: $p16^{INK4a}$ and $p19^{Arf}$), and results in increased expression of p16 and p19 [43]. As a consequence, *Ezh2* mutant mice have reduced postnatal β cell proliferation, leading to reduced β cell mass, hypoinsulinemia, and mild diabetes. Unlike wild-type mice, they also fail to regenerate β cells after their destruction with streptozotocin (STZ), resulting in lethal diabetes. Furthermore, homozygous inactivation of the *Cdkn2a* locus restores normoglycemia and β cell proliferation capacity in mice with β cell-specific deletion of the *Ezh2* gene [43]. Therefore, the suppression of *Cdkn2a* locus by H3K27me3 seems to be essential for the control of early postnatal proliferative capacity of pancreatic β cells. Notably, the *CDKN2A* locus is one of the GWAS genes linked with susceptibility to T2D in humans [6]. In addition to Ezh2, β cells also express another H3K27 methyltransferase—Ezh1 [43], and therefore a complete understanding of the role of H3K27me3 will require a full abrogation of this repressive marking system.

Another epigenetic regulator that plays an important role in the maintenance of euchromatin structure and the transcriptional programme at islet-specific genes is the histone methyltransferase Set7/9, which mediates the methylation of H3K4 to H3K4me2, a mark associated with active transcription. Within the pancreas, the gene encoding Set7/9 (*Setd7*) is specifically expressed in islets (both in α and β cells), due to an upstream, islet-specific enhancer, that binds Pdx1. Depletion of Set7/9 by siRNA in insulinoma cells and in primary mouse islets leads to repression of many genes involved in glucose-stimulated insulin secretion, including *Ins1*, *Ins2*, *Slc2a2* (Glut2), and *MafA*, in parallel with loss of H3K4me2 at these loci [44]. Interestingly, *Setd7* is not only controlled by Pdx1 at the transcriptional level but Setd7 protein also interacts physically with Pdx1 and together they promote the transcription of Pdx1 target genes [45].

Additional evidence supporting the crosstalk between transcription factors and the epigenetic machinery in maintaining the normal function of the endocrine pancreas is provided by studies performed in islets isolated from $Hnf1a^{-/-}$ mice. *Hnf1a* encodes a transcription factor (Hnf1α) that is dispensable for the normal development of the endocrine pancreas; however, when mutated it causes the most common form of MODY (MODY3). Hnf1α is essential for the normal expression of several target genes in differentiated pancreatic β cells (such as *Slc2a2* and *Pklr*, encoding the L-type pyruvate kinase) and this control is achieved by directing nucleosomal hyperacetylation at these loci [46]. In islets collected from $Hnf1a^{-/-}$ mice, the direct target genes are not only hypoacetylated but also enriched in H3K27me3 and depleted in H3K4me2 [47]. Furthermore, the position of these target genes is modified from central domains of the nucleus, which are transcriptionally active and enriched in H3K4me2 and RNA polymerase II, towards the more peripheral subnuclear domains, enriched in H3K27me3 and with reduced access to transcriptional factories [47]. These results raise the possibility that local changes in chromatin structure induced by key transcription factors such as Hnf1α may play important roles in regulating the cell-specific spatial organization of the genome.

In contrast to the genetic approaches presented above, small molecules that target chromatin-modifying enzymes allow the study of time- and dose-dependent effects of altered histone modification levels on transcription, without changing protein complexes. Importantly, these approaches have the potential to lead to novel therapies for diabetes. A recent study measured the genome-wide transcriptional effects of 28 compounds targeting HDACs ($n = 22$), DNMTs ($n = 3$) and histone lysine methyltransferases, HKMTs ($n = 3$) in pancreatic α (αTC1) and β (βTC3) cell lines [48]. α cells exposed to most of the HDAC inhibitors switch on insulin and other β cell-specific markers, implying partial loss of cell identity. Similarly to HDACs, a positive regulation of expression of β cell factors and repression of many α cell factors was observed for one HKMT inhibitor (chaetocin, that inhibits Su(var)3-9 and G9a). In β cells, a complex effect of HDAC inhibitors on insulin-secretion was observed, with some essential components of the pathway

up-regulated and others down-regulated. Moreover, β cells treated with HDAC-I inhibitors from the orthoamino anilide class (activity restricted to HDAC1, 2 and 3) upregulated α cell markers *MafB* and *Arx* and increased glucagon expression, without significant alteration of β cell markers, suggesting that HDAC-I are important for maintenance of β cell identity [48]. One of the most interesting outcomes of this study was the finding that selective compounds can be harnessed to modulate the expression of restricted gene sets in distinct cell types. For example, the G9a/GLP histone methyltransferase inhibitors BIX-01294 and UNC0638 were found to up-regulate the cholesterol biosynthesis pathway in pancreatic (both α and β), but not hepatic cells. This effect was associated with loss of H3K9me2 at the promoters of key genes involved in this pathway, such as *Hmgcs1* and *Hmgcr*, suggesting direct G9a/GLP-dependent regulation [48].

Epigenomic maps

The distribution of several epigenetic marks including histone modifications, nucleosome positioning, and DNA methylation has been characterized recently across the genome (or in defined chromosomal regions) in adult human and rodent islets, and in purified β cells.

In adult human islets, the distribution of H3K4me3 and H3K27me3 is mutually exclusive for most genes and only few loci (such as *HOX* genes and genes encoding neuronal transcription factors) have a bivalent pattern [49]. Interestingly, the correlation between the distribution of H3K4me3 at promoter regions and gene expression seems to be dependent on the promoter's CpG content. Promoters containing CpG islands are often marked by peaks of H3K4me3, regardless of their transcriptional activity (as assessed by the presence or absence of high levels of the transcriptional elongation histone mark H3K79me2 on either side of the H3K4me3 peak) [50]. However, many promoters without CpG islands (including those of genes encoding insulin, glucagon, somatostatin, islet amyloid polypeptide, pancreatic polypeptide preprotein and transthyretin) are not marked with, or show only modest levels of, H3K4me3 and H3K79me2, suggesting that alternative histone modifications or regulatory mechanisms must be responsible for the activation of these hormone-encoding genes in human islets [49–51].

A recent study has analyzed a defined class of so-called "disallowed genes", which are defined as those genes with specific repression in one tissue only, but expressed everywhere else. Cell selective repression of disallowed genes has been proposed to ensure correct tissue function [52]. Examples of disallowed genes in mouse islets include *Slc16a1*, which encodes the monocarboxylate transporter MCT1 that mediates the transport of lactate and pyruvate across the cell membranes, and *Ldha*, which encodes lactic dehydrogenase A that catalyzes the conversion of lactate to pyruvate. These genes have important roles in ATP production when oxygen supply is low, via anaerobic glycolysis. In β cells, glycolysis is exclusively aerobic, and therefore the expression of these genes is negligible. Mechanistically, the repression of these genes takes place early postnatally and is achieved, at least in part, by gain in H3K27me3 and loss of H3K9ac at these loci [52].

In purified β cells collected from adult mice, H3K27me3 also represses regulators of inappropriate developmental fates (e.g., skeletal, neural, and heart development), as well as genes whose repression is critical for their normal function or survival (for example *Fas*, a mediator of cell death that is inactive in healthy β cells but is induced during autoimmune destruction in type 1 diabetes) [36]. In contrast, adult β cell-specific genes (such as *Pdx1*, *Neurod1*, *Pax6*, *Isl1*, etc.) lack H3K27me3 and are marked instead with H3K4me3. Surprisingly, although at a global level the pattern of H3K27me3 distribution is similar with that observed in other pancreatic cell types, β cells differ by a selective absence of PcG repression at a core of genes related to neural development. This absence of H3K27me3 allows the activity in β cells of a set of genes that are characteristically expressed in neural cells [36].

Global epigenetic analyses have also allowed to map epigenetic marks at putative long-distance *cis*-acting regulatory elements and to study their associations with gene activity. FAIRE-seq (formaldehyde-assisted isolation of regulatory elements coupled with high-throughput sequencing) is a technology that enables the mapping of open chromatin sites marked by absence of nucleosomes—an evolutionary conserved indicator of regulatory activity (i.e., promoters or enhancers). When applied to purified human islets, this method led to the identification of approximately 80,000 open chromatin sites and over 3,300 islet-specific clusters of open regulatory elements (COREs) (i.e., three or more open chromatin sites separated by less than 20 kb, which are found in islets but are absent in five non-islet cell lines) [53]. Supporting their potential role in gene regulation, most COREs were associated with only one gene and these genes tended to have higher expression in islets when compared to other primary tissues [53]. Additionally, islet-selective COREs are enriched in long noncoding RNAs (lncRNAs), many of which are highly islet-specific (compared with 16 other human tissues) and important for the β cell differentiation and maturation program [54]. Another study used global distribution mapping of H3K4me1 in human islets (a histone modification that has been found to mark active enhancers and promoters [55]), and reported on approximately 35,000 intergenic peaks [49]. Genome-wide mapping of DNaseI hypersensitive sites (DHS, which mark open chromatin regions), combined with the mapping of

CTCF binding sites (an insulator protein) and H3K4me1 peaks [50] identified over 100,000 DHS in human islets, of which approximately 34,000 are distal to transcription start sites (TSS) and 47 % are islet-"specific" (i.e., not found in four different human cell lines). CTCF binds to about a fifth of these DHS. H3K4me1 is depleted at many DHS located near TSS, but is enriched at distal DHS, suggesting that the combination DHS–H3K4me1 enrichment is a good predictor for active enhancers [50]. Importantly, all of these studies have shown that many SNPs that have recently been associated with T2D through GWAS studies are located at putative regulatory elements (as identified by the criteria used in the above studies), and enhancer activities have been experimentally validated for several of these elements [50, 53, 55].

Methods that are able to identify functionally active TF binding events are essential for interpretation of genome-wide TF binding data, in particular because most transcription factors have the potential to bind thousands of loci at the sequence level, and many may bind without a functional correlate (i.e., gene targets are inactive). A recent study performed on isolated adult pancreatic islets has tested the hypothesis that Foxa2 and Pdx1-bound loci that are transcriptionally active may exhibit local enrichment in H3K4 methylation, as well as the characteristic patterns of nucleosome positioning [56]. This combined analysis found that TF-bound loci can be distinguished into three classes. The first class corresponds to transcriptionally active genes and is characterized by enrichment in H3K4me3 and a bimodal distribution of H3K4me1, with peaks on each side of the nucleosome-depleted transcription binding site. The bimodal loci that bind concomitantly Foxa2 and Pdx1 have higher levels of transcription and are more often expressed in islets. The second class of loci corresponds to genes poised for activation and are characterized by a single peak of H3K4me1 (monomodal distribution), due to the presence of H3K4me1-marked nucleosomes at the transcription binding site. The third class of loci show low levels of H3K4me1, despite the binding of Foxa2 and Pdx1 and are transcriptionally inactive. These loci reflect perhaps the ability of the TFs or that of their interacting partners, to bind to compacted chromatin (so-called "pioneer TF") [56]. Consistent with this possibility, Foxa2 is a well-established "pioneer TF" [57].

So far, there are only two published studies that analyzed the DNA methylome of the endocrine pancreas. The first study, performed in adult rat islets, was based on the HELP assay (*Hpa*II tiny fragment enrichment by ligation-mediated PCR), followed by microarray hybridization [58]. Consistent with studies performed in other tissues and species, this analysis found that hypomethylated loci are often located at CpG islands and gene promoters and, more rarely, in gene bodies and at conserved intergenic regulatory elements. In contrast, repetitive sequences tended to be constitutively hypermethylated, a pattern that is typical to all eutherian mammals [58]. The second study performed on human islets used the recently developed Infinium Methylation BeadChip arrays that allow the interrogation of the methylation status of 27,578 CpG sites corresponding to 14,475 gene promoters [59]. This analysis, performed on diabetic and non-diabetic samples, led to the identification of T2D-related differentially methylated genes (presented in a later section of this review) [59].

Chromatin interactions

The genome is highly organized inside the nucleus into a compact chromatin structure that facilitates many direct interactions between otherwise distal sites. Many of these interactions are thought to be essential for tissue-specific promoter-enhancer interactions and the coordinate transcription between genes. Two recent studies have started to map out some of these long-range interactions in pancreatic islets. In human islets, the *INS* gene interacts with a large number of loci, the majority located within 1-Mb. One of these genes is *SYT8* (which encodes synaptognamin 8), located over 300 kb away from *INS* [60]. Interestingly, this interaction is attenuated when the insulator protein CTCF is depleted by siRNA and, consequently, *SYT8* transcription is reduced. Conversely, the *SYT8-INS* interaction is more pronounced when islets are exposed to increased concentrations of glucose, by yet unknown mechanisms [60]. In support of the functional importance of this interaction for the normal function of the pancreatic β cell, siRNAs that target the *INS* promoter and induce transcriptional silencing of *INS* gene also lead to decreased *SYT8* mRNA expression, but not of other neighboring loci. Furthermore, siRNA depletion of SYT8 protein reduces glucose- and arginine-stimulated insulin secretion [60]. Therefore, this study demonstrates that, in human islets, the *INS* promoter not only controls insulin transcription but also orchestrates key steps in insulin secretion through long-range interactions with the *SYT8* locus.

In rats and humans, the *Hnf4a* gene (which encodes hepatocyte growth factor 4 alpha, required for pancreatic β cell differentiation and glucose homeostasis) has two alternative promoters, proximal P1 and distal P2, and an enhancer region located in between. Transcriptional activity of *Hnf4a* in islets is restricted to the distal P2 promoter, which is enriched in H3 acetylation (H3Ac) and H3K4me3. The intragenic enhancer region also has an open chromatin pattern (enriched for H3Ac and H3K4me1) in both species. The *Hnf4a* enhancer region interacts with P2 promoter specifically in rat pancreatic islets and this interaction is important for the normal transcriptional activity of the P2 promoter [61].

Epigenetic reprogramming of cellular memory directed towards endocrine cell function

A major goal in the treatment of diabetes is to regenerate the capacity of the β cell to produce insulin. The search for β cell replacement therapies is currently focused on the expansion of β cell mass in vivo and generation of β cells in vitro for transplantation. With our increasing knowledge of the basic mechanisms that govern the development of the endocrine pancreas, including that of the "epigenetic programme", new potential therapies for diabetes are emerging. Strategies that are based on principles of reprogramming differentiated cells types towards insulin-producing cells are of particular promise in the context of regenerative medicine and clinical practice of diabetes.

Human-induced pluripotent stem cells (iPSCs) have recently been obtained from cultured human β cells transduced in vitro with retroviral vectors encoding the four pluripotency- iPS-inducing factors OCT4, SOX2, KLF4, and cMYC [62]. The efficiency of human β cell reprogramming was found to be about 25 times lower compared to that of fibroblast cells, which could be attributed to a more differentiated state of the β cells [61]. β cell-derived iPSCs (βiPSCs) maintain a partially open chromatin structure at the promoter region of *INS* and *PDX1* genes, with high levels of H3Ac as well as low levels of DNA methylation at the *PDX1* promoter, patterns that contrast with those observed in regular ES cells, or in iPSC derived from fibroblasts. βiPSCs also have an increased ability to differentiate into insulin-producing cells, both in vivo and in vitro, compared to ES cells and isogenic non-β iPSCs [62]. All together, these results suggest that βiPSCs maintain an epigenetic memory at key β cell genes. The maintenance of the epigenetic memory inherited from the donor cells may explain, at least in part, the skewed differentiation potential towards insulin-producing cells. These findings may be very useful when considering replacement therapy using reprogrammed cells.

An alternative method for generating insulin-producing cells is to differentiate multipotent or pluripotent cells under appropriate in vivo or in vitro conditions. Various cell types have been reported to differentiate into insulin-producing cells, including hepatic oval cells [63], splenocytes [64], ES cells [65, 66], and bone marrow-derived stem cells (BMSC) [66]. Human ES cells treated with the HDAC inhibitor sodium butyrate and with activin A generate definitive endoderm cells, which then can be further differentiated into insulin-producing islet-like clusters with the aid of specific growth factors [66]. Moreover, BMSC treated with the HDAC inhibitor TSA (trichostatin A) and cultured subsequently in media containing GLP1 (glucagon-like peptide 1) and high concentrations of glucose are efficiently differentiated into insulin-producing cells and other endocrine pancreas cell types [67].

In vivo reprogramming of pancreatic cell types has also been documented in a number of studies involving manipulations in mice: adult pancreatic acinar cells were shown to convert into β cells upon viral-mediated re-expression of Pdx1, Ngn3, and Mafa [68], endocrine precursor cells, as well as mature α cells, adopt a β cell destiny upon ectopic expression of Pax4 [69], α into β cell conversion is observed after severe ablation of β cells using diphtheria toxin [70], β into α cell conversion was found upon deletion of Dnmt1 or Dnmt3a in β cells [41, 42] and postnatal α to β cell reprogramming was described to occur upon enforced Pdx1 expression in Ngn3 + endocrine precursor cells [71].

Epigenetic mechanisms are likely to play essential roles in these trans-differentiation processes in vivo. However, supporting evidence is currently rather limited. Direct evidence comes from the studies mentioned above that show that loss of DNA methylation allows β cells to spontaneously reprogramme and re-direct into α cells [41, 42]. Less clear is how pancreatic lineage-specific transcription factors interact with the epigenetic programme to drive cellular reprogramming. The transcription factor Pdx1 acts as an autonomous reprogramming factor of α to β cell conversion [71], while being implicated in epigenetic modifications, via interaction with the histone methyltransferase Set7/9, a Pdx1-responsive factor that enhances chromatin accessibility and transcription of β cell genes. It has been proposed that exogenous Pdx1 acts as an initiator of the epigenetic reconfiguration from α to β by influencing the recruitment of Set7/9 to specific β cell-specific loci. Learning how transcription factors influence the epigenetic landscape of islet genes will be essential to fully understand cellular reprogramming in vivo and in vitro, with important implications for regenerative medicine protocols.

Environmental epigenetic programming of the endocrine pancreas

Epigenetic alterations induced during early development

Epidemiological studies have revealed strong relationships between patterns of early growth and subsequent risk of T2D development in adulthood (reviewed in [72, 73]). The first study to link birth weight to T2D was carried out on a group of men living in Hertfordshire in the UK, who at the time of study had a mean age of 64 years but for whom birth-weight records were available [74]. This study revealed that those men who had the lowest birth weight were six times more likely to have either impaired glucose tolerance or T2D, than those men who were heaviest at birth [74]. Additionally, individuals who were exposed in utero to

the Dutch famine (which occurred in the western part of the Netherlands at the end of World War II) and were born as thin babies, were more likely to have become glucose intolerant when studied in their fifties [75]. These observations identified the phenomena of developmental programming of T2D, without explaining the mechanisms involved [73]. To provide mechanistic insights, several animal models of early programming have been established, ranging from rodents to sheep and non-human primates. In these models, different types of in utero insults, including maternal sub-optimal nutrition occurring during different periods of early development were established, from protein restriction and calorie restriction to uterine artery ligation, gestational diabetes, and a high-fat diet [76]. The studies performed in animal models have highlighted a common principle: the offspring demonstrate β cell dysfunction, irrespective of the type of suboptimal nutrition. The cellular and molecular mechanisms that explain intrauterine programming of the endocrine pancreas remain unclear, although dysregulation of gene expression through epigenetic modifications is gaining momentum [11, 25] and will be discussed in detail in the section below.

In the rat, uteroplacental insufficiency induced by maternal uterine artery ligation at day 18 of gestation, leads to chromatin remodeling at the promoter of *Pdx1* gene in the pancreatic islets, as early as fetal day 21 [77]. The first epigenetic alterations found at the *Pdx1* promoter are a significant loss of H3Ac and H4Ac due to local enrichment of the histone deacetylase HDAC1, associated with enrichment of the co-repressor protein Sin3A and decreased binding of the transcriptional activator USF1. Together, these epigenetic changes lead to reduced *Pdx1* transcription. After birth, the initial epigenetic changes are followed by a progressive decrease in the active histone mark H3K4me3 and a significant increase in the repressive mark H3K9me2. After the onset of diabetes in adulthood, at 6 months of age, the CpG island at the *Pdx1* promoter becomes methylated, likely due to increased local recruitment of the DNA methyltransferases Dnmt1 and Dnmt3a, leading to permanent silencing of the locus [77]. Using the same experimental model, a genome-wide DNA methylation analysis was performed in 7-week-old rat pancreatic islets, which led to the identification of significant changes at ~1,400 loci. No overall tendency towards decreased or increased methylation was found between offspring of ligated mothers compared to non-ligated controls [58]. Interestingly, most DNA methylation alterations were located at conserved non-coding intergenic sequences, which may represent important *cis*-regulatory sites influencing local gene expression. Importantly, these epigenetic changes precede the appearance of T2D and, thus, the affected loci may represent novel candidates for mediating programming effects [58].

Rats exposed to an isocaloric low-protein (LP) diet containing 8 % protein (instead of 20 % for the control—C diet) during pregnancy and lactation, give birth to smaller offspring that have normal glucose tolerance as young adults. However, the male LP offspring undergo an age-dependent loss of glucose tolerance and develop a phenotype similar to human T2D by 17 months of age [78]. The development of diabetes in LP rat offspring is associated with progressive silencing of the *Hnf4a* gene [61]. Remarkably, the transcriptional repression of the *Hnf4a* gene in LP islets is brought about by the specific epigenetic silencing of an intragenic enhancer element, which loses active histone marks such as H3Ac and H3K4me1 and gains repressive marks such as H3K9me2, without significant DNA methylation changes. This epigenetic silencing of the enhancer element leads in turn to a weaker interaction with the upstream P2 promoter, determining a permanent reduction in *Hnf4a* expression [61]. Exposure to the LP diet during early development also influences the dynamics of age-related epigenetic changes for several histone marks, most remarkably the rate at which H3K27me3 is enriched at the P2 promoter and the enhancer region [61].

Recent studies suggest that epigenetic alterations in the offspring's endocrine pancreas can also be induced by suboptimal paternal nutrition. Chronic paternal exposure to a high-fat diet (HFD) in rats leads to early impairment of glucose tolerance in F1 female offspring that worsens with time [79]. Moreover, these F1 females have reduced β cell mass and altered islet expression of many genes involved in glucose metabolism. Importantly, the gene that has the highest level of transcriptional upregulation in F1 HFD females (*Il13ra2*) also had reduced DNA methylation at a CpG dinucleotide located in the promoter area, which overlaps a putative binding site for the transcriptional repressor NF-X, a protein that binds specifically to methylated DNA [79].

The studies presented in this section provide evidence that epigenetic alterations occurring during developmental programming of the endocrine pancreas are induced, directly or indirectly, by sub-optimal environmental conditions. Together with similar epigenetic analyses in organs such as liver [80–82], adipose tissue [83], and brain [84, 85], these studies suggest that histone marking systems are more often altered by environmental cues, while changes in DNA methylation (an epigenetic mark considered more stable) are less pronounced. Current studies also suggest that environmentally induced epigenetic alterations affect not only promoters of protein-coding genes [reviewed in 10, 11] but also transposable elements such as the IAP element at the A^{vy} locus [86], and regulatory elements such as the enhancer region located downstream the *Avp* (arginine vasopressin) gene [85], the intragenic *Hnf4a* enhancer [61, 87], or the intergenic CpG island located upstream the *Ppara* gene [82].

Age-associated epigenetic alterations

Aging is a major determining risk factor for glucose intolerance and T2D in humans [88, 89]. A progressive decline in β cell function in the elderly is a key contributing factor to the pathophysiology of T2D, in addition to other widely recognized aging-associated changes such as decreased physical fitness and increased abdominal adiposity [90, 91]. Similar age-associated impairment of β cell function has been demonstrated in rodents [92, 93]. The mechanisms that underlie the association between aging and increased risk of T2D are unknown. Cellular senescence (the phenomenon by which normal diploid cells lose the ability to divide) is known to contribute significantly to the aging phenotype, and this will be discussed in detail in the context of diabetes risk in the sections that follow. Molecular hallmarks of cellular senescence include upregulation of the cyclin-dependent kinase inhibitor $p16^{INK4a}$, accumulation of oxidative damage and telomere shortening [94].

Several studies are suggestive of a higher propensity of pancreatic isles to cellular senescence when compared with other tissues [95–97]. The expression of the cell-cycle repressor $p16^{INK4a}$ increases 14-fold in the pancreatic islets of 64 to 80-week-old mice compared with 12 to 14-week-old mice, and this upregulation constrains β cell proliferation with aging. However, $p16^{INK4a}$ expression remains unchanged in the exocrine pancreas [95]. Additionally, CENPA, a protein member of the histone family found in the nucleosomes of active centromeres and required for the correct chromosome segregation during cell division, also declines with age in islets, but not in acinar cells. The decline of CENPA protein levels is more striking in islets collected from humans than from mice. Interestingly, mRNA levels in human islets are not correlated with the age of the donors, suggesting that the decline observed at the protein level is the result of post-translational mechanisms [96]. Another line of evidence that supports a higher propensity to cellular senescence is the observation that pancreatic β cells have very low expression levels of antioxidant enzymes such as catalase and glutathione peroxidase compared with other cell types [97]. This renders them more sensitive to the action of reactive oxygen species (ROS). Finally, telomere length seems to have a stronger phenotypic impact on pancreatic islets compared with other tissues, such as fat. Accordingly, mice deficient for the *Terc* gene (encoding the RNA component of the telomerase complex), which have shorter telomeres and reduced viability with age, are glucose intolerant because of reduced islet size and exhibit altered glucose-stimulated insulin secretion from the β cells. However, the body fat content, the energy expenditure and the insulin sensitivity remain unaltered [98]. This high susceptibility of the endocrine pancreas to cellular senescence may be an important contributing factor for development of T2D in elderly individuals. In this context, it is important to mention that pancreatic islets of rats exposed to an LP diet during gestation, show accelerated telomere shortening and increased markers of cell senescence, alterations that are associated with an increased risk for T2D [99].

Recent studies have found ample evidence for epigenetic perturbations associated with aging in a variety of mammalian tissues. These include loss or gain of DNA methylation in a sequence-specific manner [100–106], gain or loss of 5-hydroxymethylcytosine in specific gene bodies and repetitive DNA sequences [107, 108], alterations in many histone marks [100, 109–111], accumulation of specialized domains of facultative heterochromatin called senescence-associated heterochromatin foci (SAHF) [112, 113], and loss of core histones [114, 115]. Despite the increasing catalogue of epigenetic alterations with aging, the involvement of such mechanisms in the decline of the function of the endocrine pancreas has not been thoroughly studied. In mouse islets, the expression of the histone methyltransferase Ezh2 declines rapidly in juvenile life, and then slowly in adulthood, similar to the tempo of the age-dependent decline of H3K27me3 repression at the *Cdkn2a* locus [43]. Importantly, the expression of the *EZH2* gene is also downregulated with aging in human islets [43] and this decline is controlled in the two species by a conserved pathway regulated by PDGF–PDGFR-α (platelet-derived growth factor receptor alpha), whose activity also weakens with aging in pancreatic β cells due to an age-dependent β cell PDGFR-α loss [116]. In rat islets, expression of the *Hnf4a* gene is downregulated with age and this expression change is associated with a modest increase in DNA methylation at the P2 promoter and, more notably, with substantial changes in levels of specific histone marks. These alterations include loss of active histone marks at P2 promoter (H3Ac and H3K4me3) and the intragenic enhancer region (H3Ac and H3K4me1) and accumulation of repressive histone marks (H3K9me2) at both regulatory regions. The most striking histone alteration is however the accumulation of H3K27me3 throughout the locus [61]. The accumulation of H3K27me3 at the *Hnf4a* locus in old rat islets is likely to be mediated by the Ezh1 histone methyltransferase, whose activity remains high with age [117]. In further support for the potential involvement of epigenetic alterations in the age-related loss of plasticity, a recent study in mouse islets identified differential expression of mRNAs encoding for chromatin remodeling components, such as downregulation of *Smarca1* with aging [118]. *Smarca1* is a member of the SWI/SNF family of proteins that have been strongly implicated in the aging process [118].

Epigenetic alterations in the endocrine pancreas of diabetic patients

Although epigenetic mechanisms have clear roles in regulating normal development and function of the endocrine pancreas, the evidence for a direct link between epigenetic alterations in these cells and the pathogenesis of human diabetes is still fairly limited. One of the earliest examples that strongly suggested such a possibility was provided by the study of patients with transient neonatal diabetes mellitus (TNDM), a disease associated with intrauterine growth failure, dehydration, hyperglycemia, and failure to thrive, which usually resolves spontaneously by 6 months of age [119]. Genetic evidence demonstrated that TNDM is often associated with increased expression of the imprinted gene *ZAC/PLAGL1* (which encodes a zinc finger protein that promotes apoptosis and cell-cycle arrest) located on human chromosome 6q24 and is expressed from the paternal allele only [119, 120]. In some TNDM patients, uniparental disomy of the paternal 6q24 chromosomal region results in double dosage of *PLAGLI*. However, many patients with normal karyotype exhibit loss of DNA methylation at a CpG island that partially overlaps *PLAGL1* and is thought to control the imprinted expression in this chromosomal region [121, 122]. Importantly, *PLAGL1* is expressed in developing human pancreatic β cells and declines drastically after the second trimester [123]. Furthermore, transgenic mice expressing multiple copies of the human TNDM imprinted locus (i.e., *PLAGL1* and *HYMAI* genes), exhibit impaired development of the endocrine pancreas and altered glucose homeostasis [124].

Recent gene candidate studies led to the identification of several epigenetic alterations in pancreatic islets of T2D patients. The expression of the *PPARGC1A* gene, a transcriptional coactivator that regulates genes involved in energy metabolism, is decreased in pancreatic islets of patients with T2D and this altered expression is associated with increased levels of DNA methylation at the promoter region [125]. DNA methylation is also increased at the *INS* promoter and at the distal *PDX1* promoter and enhancer in islets of T2D patients compared with non-diabetic controls, and is inversely correlated with mRNA levels [126, 127]. Interestingly, DNA methylation at the *INS* promoter and distal *PDX1* promoter correlates positively with levels of glycosylated hemoglobin (HbA1c—a marker of poor glycemic control) in patients with T2D and the in vitro exposure of rat INS1 cells to high concentrations of glucose for 72 h also increases DNA methylation at the *INS1* and *PDX1* promoters [126, 127]. However, it remains unclear whether the altered DNA methylation at the *INS* and *PDX1* promoters in patients with T2D are causative of the disease or secondary effects to chronic hyperglycemia.

The most comprehensive study so far that addresses the role of epigenetic alterations in the pathogenesis of T2D in humans has recently uncovered 254 genes with differential DNA methylation in diabetic islets compared to controls [59]. Strikingly, the vast majority of these genes show decreased DNA methylation in T2D islets, have promoters with low or intermediary CpG density, and a subset of them also have significant and opposite differential mRNA expression between control and diabetic islets. Of significance, none of the DNA methylation alterations identified in islets were found in blood cells of diabetic patients or in non-diabetic islets exposed to high glucose in vitro, suggesting that they may play a role in the pathogenesis of the disease by acting specifically in islets. Accordingly, RNAi knockdown of eight selected differentially methylated genes in INS-1E rat insulin-secreting cells modified their function or survival [59].

Recent evidence suggests that the occurrence of epigenetic alterations in the endocrine pancreas might be influenced by underlying DNA sequence variants. In this context, some of the recently identified GWAS alleles that increase T2D risk and are located at putative islet-specific enhancer elements seem to associate local chromatin alterations. For example, human islet samples heterozygous for the C/T SNP rs7903146 (a *TCF7L2* intronic variant strongly associated with T2D) show allelic imbalance in FAIRE-Seq signals and abnormal enhancer activity, indicating that this genetic variation alters *in cis* the organization of the local chromatin [50, 53]. The impact of genetic variants on levels of DNA methylation and diabetes risk has also been evaluated in peripheral blood obtained from T2D patients and healthy controls. For example, the C/T SNP rs2334499 located in the imprinted region of human chromosome 11p15 associates with an increased risk for T2D only when the risk allele (T) is paternally inherited and is protective when maternally transmitted [128]. Importantly, rs2334499 is located within a parent-specific differentially methylated CTCF-binding site and the risk allele associates with decreased DNA methylation in the peripheral blood [128]. Another recent genome-wide DNA methylation survey identified several other differentially methylated sites in the vicinity of SNPs associated with the disease in previous GWAS studies, such as *THADA*, *JAZF1*, *SLC30A8*, *TCF7L2*, *KCNQ1*, and *FTO* [128]. The top-ranking association was for a CpG site located in the first intron of the *FTO* gene that showed a small (3.35 %) but highly significant hypomethylation in cases relative to controls [129]. Importantly, although the T2D-associated SNP in the *FTO* region influences the levels of DNA methylation, the hypomethylation in the T2D group is independent of the sequence polymorphism and persists in individuals that carry the risk alleles. Furthermore, a prospective study in an independent population cohort showed that young individuals (30 years old) that develop impaired glucose metabolism by the age of 43 have already significant hypomethylation relative to

those that stay healthy, suggesting that the epigenetic alteration precedes the metabolic changes [129]. The identification of diabetes-associated DNA methylation alterations in a tissue (e.g., blood) that is not directly involved in insulin secretion or action further suggests their occurrence in early stages of development. In summary, together these studies provide evidence for both sequence-influenced and sequence-independent DNA methylation variations at loci that predispose to T2D.

Molecular mechanisms of environmentally induced epigenetic states

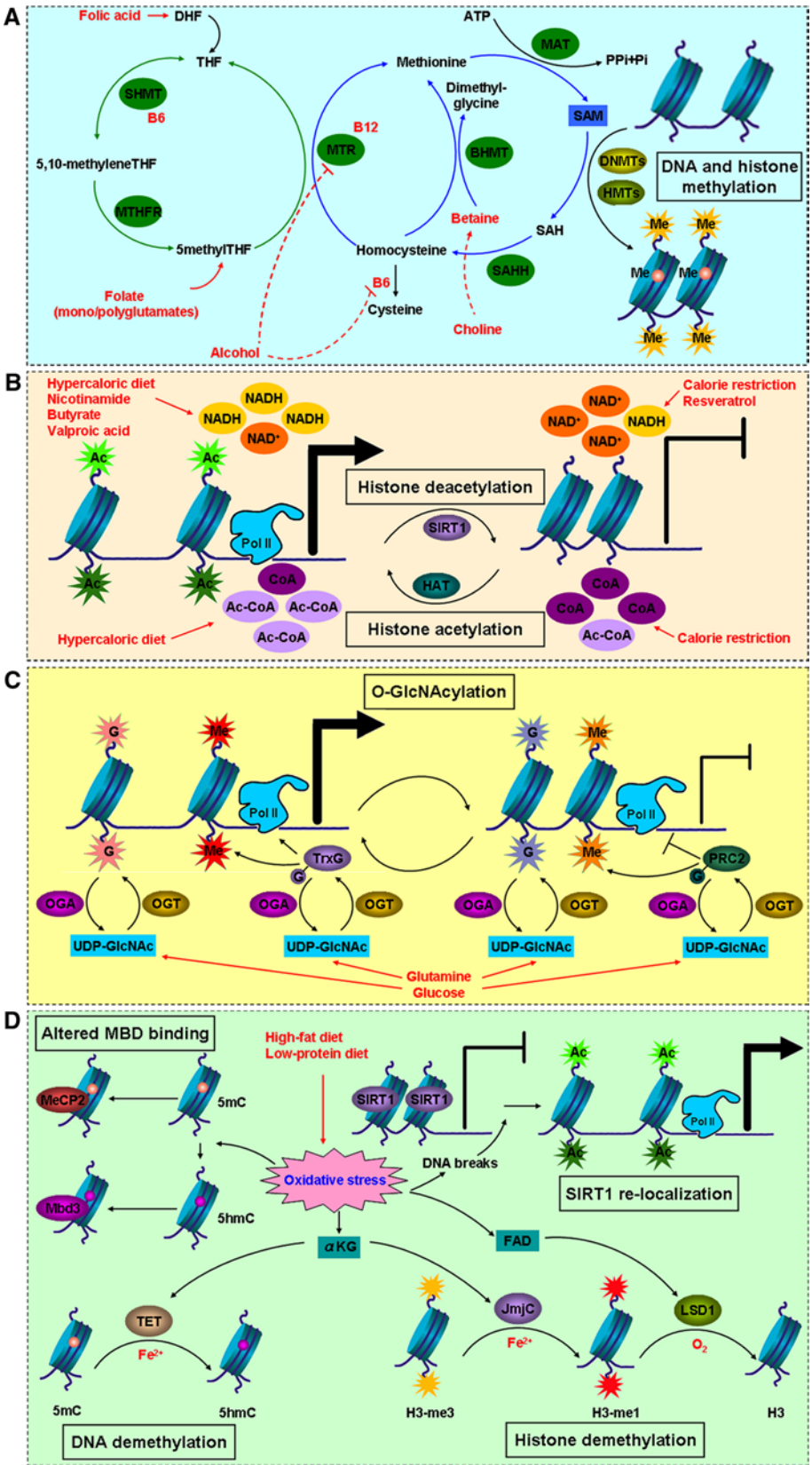
An emerging "quest" in epigenetics research is to understand how the environment shapes the epigenome through signaling pathways from the cell surface to the DNA organized into chromatin. While it is clear that environmental stimuli and the metabolic status of a cell influence the epigenome and hence the transcriptional programme, the molecular pathways remain largely elusive. In broad terms, epigenetic states will be directly influenced by alterations in the abundance or efficacy of the "writers" and "erasers" of epigenetic information (i.e., enzymes and co-factors that add or remove epigenetic marks, respectively) or by altering the availability of their direct substrates (i.e., levels of methyl, acetyl, phosphate or UDP-GlcNAc [uridine diphosphate *N*-acetylglucosamine] groups) [130, 131]. Therefore, changes in the levels of cellular metabolites will influence the epigenome and induce heritable effects. Moreover, many enzymatic reactions that are involved in the establishment or the erasure of epigenetic marks are dependent on metabolic coenzymes such as *S*-adenosylmethionine (SAM), nicotinamide adenine dinucleotide (NAD), acetyl-coenzyme A (acetyl-CoA), flavin adenine dinucleotide (FAD), and α-ketoglutarate (αKG) (reviewed in [130]). Furthermore, the biosynthesis of these coenzymes requires adenosine-5′-triphosphate (ATP) [132–134], with ATP production being tightly linked with the metabolic state of the cell. In this section, we summarize recent advances that link cellular metabolism and oxidative stress to epigenetic signaling and discuss their relevance to endocrine pancreatic function.

SAM and the methylation of DNA and histones

One-carbon metabolism controls the amount of available SAM, which is the major biological methyl donor in cells (Fig. 2a). To generate SAM, homocysteine is first methylated to methionine by transferring a methyl group from 5-methyl-tetrahydrofolate or betaine. Methionine is activated enzymatically to SAM, which is then converted to *S*-adenosylhomocysteine (SAH) upon donating the methyl group to methyltransferases such as DNMTs (DNA methyltransferases) and HMTs (histone methyltransferases) that then use the methyl group to methylate DNA and histones, respectively. SAH is hydrolyzed to homocysteine, which can then be reused to form methionine or undergoes the transsulfuration pathway to form cysteine. Cysteine is ultimately transformed into a variety of sulfur-containing molecules, including glutathione (GSH), an important antioxidant that prevents the damage to important cellular components caused by ROS (Fig. 2a) [132, 135, 136]. The major external sources of methyl groups are dietary folate, methionine, and choline, while vitamins B6 and B12 are required as cofactors for key enzymes that participate in the one-carbon metabolism (Fig. 2a) [132, 135]. Alcohol intake can reduce SAM production by altering the conversion of homocysteine to methionine and that of homocysteine to cysteine (Fig. 2a) [136].

The impact of dietary manipulations of methyl donors on the epigenome of the endocrine pancreas has not been reported so far, although the consequences of methyl-deficient diets on glucose homeostasis in rats have been described recently. Accordingly, a maternal diet deficient in methyl donors (90 % reduction in levels of methionine, folate, and choline) led to increased neonatal lethality, low birth weight, and a 46 % reduction in the offspring's endocrine pancreas mass relative to controls, which was associated with a mild impairment of glucose tolerance and insulin secretion [137]. When the methyl-deficient diet was fed post-weaning only, a reduction in body weight and in

Fig. 2 Potential molecular mechanisms for environmentally induced epigenetic states (adapted from [130–136]; see also text for details). For all panels, the environmental factors (diet, drugs, etc.) are labeled in *red*. **a** Alterations of the one-carbon metabolism. *SHMT* serine hydroxymethyl-transferase, *MTHFR* methylene-tetrahydrofolate reductase, *MTR* 5-methyl-tetrahydrofolate-homocysteine methyltransferase, *BHMT* betaine-homocysteine methyltransferase, *SAHH* *S*-adenosyl-homocysteine hydrolase, *MAT* methionine-adenosyl methyltransferase, *DHF* dihydrofolate, *THF* tetrahydrofolate, *SAM* *S*-adenosyl-methionine, *SAH* *S*-adenosyl-homocysteine, *ATP* adenosine-5′-triphosphate, *DNMTs* DNA methyltransferases, *HMTs* histone methyltransferases, *Me* methylation. **b** Alterations of the histone deacetylase SIRT1 (sirtuin 1) and histone acetyltransferase (HAT) activities induced by factors that change the intracellular NAD^+/NADH and Acetyl-CoA/CoA ratios: a high NAD^+/NADH ratio enhances SIRT1 activity, with consequent histone deacetylation, while high levels of acetyl-CoA (Ac-CoA) stimulate HAT activity, leading to histone acetylation (Ac). **c** Epigenetic alterations induced in response to nutrient changes via the hexosamine biosynthetic pathway and *O*-GlcNAcylation of histones and histone modifiers. *UDP-GlcNAc* uridine diphosphate *N*-acetylglucosamine, *OGT* *O*-GlcNAc transferase, *OGA* *O*-GlcNAcase, *G-O*-GlcNAcylation, *PCR2* PcG complex 2, *TrxG* Trithorax group proteins, *Me* methylation. **d** Epigenetic alterations induced by increased oxidative stress. *MBD* methyl binding proteins (such as MeCP2 and Mbd3), *5mC* 5-methylcytosine, *5hmC* 5-hydroxymethylcytosine, *αKG* α-ketoglutarate, *FAD* flavin adenine dinucleotide, *TET* ten-eleven translocation (a DNA demethylase), *JmjC* Jumonji domain-containing (histone demethylases), *LSD1* lysergic acid diethylamide (histone demethylase), *Pol II* polymerase II


A
Folic acid
DHF
THF
SHMT
B6
5,10-methyleneTHF
MTHFR
5methylTHF
Folate
(mono/polyglutamates)
Alcohol
B12
MTR
Methionine
ATP
MAT
PPi+Pi
Dimethyl-
glycine
BHMT
SAM
Betaine
SAH
Homocysteine
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B6
Cysteine
Choline
DNMTs
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methylation
Me
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Hypercaloric diet
Nicotinamide
Butyrate
Valproic acid
NADH
NAD+
Ac
Pol II
CoA
Ac-CoA
Hypercaloric diet
Histone deacetylation
SIRT1
HAT
Histone acetylation
Calorie restriction
Resveratrol
Calorie restriction
C
O-GlcNAcylation
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Pol II
TrxG
PRC2
OGA
OGT
UDP-GlcNAc
Glutamine
Glucose
D
Altered MBD binding
High-fat diet
Low-protein diet
MeCP2
5mC
Mbd3
5hmC
SIRT1
Oxidative stress
DNA breaks
SIRT1 re-localization
αKG
FAD
TET
Fe2+
JmjC
LSD1
O2
5mC
5hmC
H3-me3
H3-me1
H3
DNA demethylation
Histone demethylation

pancreatic endocrine mass (−40 %) was also observed, however this was associated with improved glucose tolerance despite decreased insulin secretion [137]. Whether these observed phenotypes are causally linked to an altered epigenome of the endocrine pancreas is unknown. However, altered epigenetic states resulting from methyl-enriched or methyl-deficient diets are well documented in rodent models [86, 138] and humans [139].

Acetyl-CoA/CoA and NAD^+/NADH ratios and histone acetylation

An exciting hypothesis that has recently been put forward proposes that histone-modifying enzymes act as sensors of metabolic changes within a cell and convert those changes into stable patterns of gene expression. A central role for histone acetylation and methylation in modulating metabolic circuits has been clearly demonstrated, at the level of whole body physiology, in studies of knock-out mice deficient for specific histone-modifying enzymes, which develop a range of metabolic phenotypes from lethal hypoglycemia to obesity [134, 140]. At the cellular level, important inroads were made with the finding of novel mechanisms that link glucose metabolism to chromatin modification and global transcriptional control via the enzyme ATP-citrate lyase (ACL) and production of acetyl-CoA [141]. Accordingly, it has been shown that ACL-generated acetyl-CoA is an essential co-factor for HATs by serving as the acetyl group donor. Of note, this pathway is up-regulated by glucose and insulin signaling via phosphorylation by AKT, leading to increases in acetyl-CoA [141].

Another significant advance in our understanding of the relationship between cellular metabolism and the epigenetic programme relates to the discovery that chromatin regulators, such as the sirtuins, are able to "sense" intracellular NAD^+/NADH ratios (Fig. 2b). Sirtuins belong to class III HDACs that are dependent on local concentrations of the coenzyme nicotinamide adenine dinucleotide (NAD^+). NAD^+ accepts electrons from other molecules, as it is reduced to NADH. This change in the redox status, particularly in the mitochondria, is essential for ATP production. NAD^+/NADH ratios are thought to be "sensed" by sirtuins to regulate their HDAC activity. Interestingly, glucose, in addition to increasing acetyl-CoA and HAT activity, is also thought to decrease NAD^+ (relative to NADH). When glucose is abundant, Sirt1 activity is relatively low, whereas low glucose and periods of prolonged fasting and energy limitation trigger NAD^+-induced Sirt1, which deacetylates histones and down-regulates protein synthesis [142, 143] (Fig. 2b). Therefore, glucose seems to induce global histone acetylation in a dual mechanism involving acetyl-CoA and NAD^+ intracellular levels (Fig. 2b). There is also cross-talk between the NAD^+-induced sirtuins and the acetyl-CoA pathways, as sirtuins have been shown to regulate acetyl-CoA synthetases [144]. Moreover, the relative activity of SIRTs, as mentioned above, may themselves modulate intracellular levels of NAD^+, hence establishing a dynamic metabolic state [145].

The enzymatic activity of specific chromatin remodelers, such as SIRTs, must also be seen in the context of their recruitment to specific chromosomal domains via their interaction with DNA binding domains [146]. Recently, Katada et al. [146] proposed a hypothetical model whereby the concentration of metabolites and chromatin remodeler enzymes may vary within chromatin microdomains or "niches". A niche with high levels of NAD^+ would lead to the activation of HDACs of the sirtuin class, resulting in deacetylation of substrates and transcriptional repression (a so-called inactive "niche"). On the other hand, high levels of acetyl-CoA would facilitate acetylation of histones and nonhistone proteins in transcriptional "hubs", leading to activation of gene expression (or active "niche") [146]. These local differences would be dictated by local trapping of essential metabolic enzymes and would require immediate replenishment of the key metabolites after their usage in modifying chromatin. This attractive model predicts that specific domains would be insulated against spikes of metabolic changes, but also that constitutive heterochromatin is much less sensitive to metabolic alterations than euchromatic regions [146].

Metabolic changes in the cell are ultimately dictated by the cellular environment and the extracellular concentration of nutrients. The environment (e.g., diet) is a source of a wide range of HDAC/SIRTs inhibitors, both naturally occurring and synthetic (Fig. 2b), which will have a more direct impact on epigenetic states (reviewed in [128]). Examples of such inhibitors include, nicotinamide, a common dietary component (found in meat, fish, and flour), butyrate, resulting from the microbial fermentation of fibers in the gut, organosulphuric compounds (such as diallyl disulfide found in garlic and sulforaphane found in cruciferous vegetables) or valproic acid, a drug used to treat neuronal disorders [130, 133, 136]. Although naturally occurring inhibitors are not as potent as synthetic ones, we can be chronically exposed to high levels of these inhibitors depending on our diet. For example, butyrate, produced in the colon by bacterial fermentation of dietary fibres, is the preferred energy source of colonocytes, rather than glucose, and it plays a key role as an epigenetic HDAC inhibitor, as well as playing roles in maintaining energy homeostasis to prevent autophagy [147]. Diet can also influence total levels of NAD because its de novo synthesis utilizes amino acids, as well as vitamin B3 (niacin) as building blocks. Interestingly, exposure of rat insulin-secreting BRIN-BD11 cells to nicotinamide (the amide of vitamin B3) and sodium butyrate resulted in reductions of cell growth, insulin content, and basal insulin secretion, as well as loss of β cell glucose sensitivity [148]. In

contrast, the activity of SIRTs is enhanced by resveratrol, a natural polyphenolic compound found in plants, fruits, and red wine, as well as in response to calorie-restricted diets (Fig. 2b) [136]. Treatment of insulin-secreting INS-1E cells and human islets with resveratrol led to an increased glucose-stimulated insulin secretion through a SIRT1-dependent mechanism. Importantly, these effects were correlated with up-regulation of key genes for β cell function, such as *Glut2*, *Gk*, *Pdx1*, *Hnf1a*, and *Tfam* [149]. These results suggest that SIRT1 may function as a sensor of environmental and metabolic changes in insulin-secreting cells, but the precise mechanisms remain largely unknown.

The hexosamine biosynthetic pathway and *O*-GlcNAcylation of histones and histone modifiers

Glucose is transported inside the cell where it is rapidly phosphorylated to glucose-6-phosphate, and next enters the glycolysis pathway to provide energy. However, it is estimated that 2–5 % of glucose is directed into the hexosamine biosynthetic pathway (HBP) to promote protein glycosylation. The final step of the HBP is the formation of uridine-diphosphate (UDP)-*N*-acetylglucosamine (GlcNAc) (UDP-N-GlcNAc for short) and other nucleotide hexosamines, which are major substrates for glycosylation of proteins. UDP-GlcNAc is utilized, amongst other enzymes, by the *O*-GlcNAc transferase (OGT) in the nucleus and cytoplasm for the addition of GlcNAc at serine (S) and threonine (T) residues of target proteins (*O*-GlcNAcylation) [131]. Addition and removal of *O*-GlcNAc by OGT and *O*-GlcNAcase (OGA), respectively, dynamically alters many intracellular proteins, including the four core nucleosome histones, at specific amino acids residues: H4S47, H2AT101, H2BS36, H2BS112 and H3S10. The impact of histone *O*-GlcNAcylation is very complex, being associated with both transcriptional repression and activation. For example, *O*-GlcNAc modification at H3S10 is associated with both active chromatin (marked by H3K4me3) and repressed chromatin (marked by H3K9me3) [150] and *O*-GlcNAc modification at H2BS112 facilitates monoubiquitylation of neighboring H2BK120 (mark associated with transcriptional elongation) [151]. Interestingly, OGT itself is part of the key chromatin regulatory complex PcG and, among different PcG proteins, polyhomeotic is glycosylated by OGT in vivo [152]. *O*-GlcNAc is also essential for the function of the TrxG complex component MLL5, a histone lysine methyltransferase [153] (Fig. 2c).

Numerous lines of evidence demonstrate that *O*-GlcNAcylation is involved in the regulation of β cell function. For example, OGT is highly enriched in pancreatic β cells and the diabetes inducing drug STZ inhibits OGA [154]. Moreover, hyperglycemia leads to the rapid and reversible accumulation of *O*-GlcNAc specifically in β cells in vivo. Animals pretreated with STZ also accumulate *O*-GlcNAc in their β cells when hyperglycemic, but this change is sustained upon re-establishment of euglycemia [155]. Young (3–4 months of age) transgenic mice that over express OGA in the pancreatic β cells have reduced levels of circulating insulin and lower insulin content in the islets and are hyperglycemic during a glucose tolerance test. However, in older mice (8–9 months of age) the glucose tolerance is no longer impaired. This is associated with increased serum insulin, islet insulin content, and insulin mRNA in the OGA transgenic mice [156]. Moreover, the human gene encoding OGA (*MGEA5*—meningioma expressed antigen 5) is a diabetes susceptibility locus in the Mexican-American population that has a high incidence of obesity and diabetes [157]. Finally, in pancreatic β cells, glucose-stimulated expression of GPR40 (a G protein-coupled receptor that plays a major role in the regulation of insulin secretion by fatty acids) is due to PI3 K-dependent *O*-GlcNAcylation of Pdx1 and consequent increased binding of Pdx1 at GPR40 promoter [158]. Although no direct evidence exists yet, one may speculate that abnormal *O*-GlcNAcylation of chromatin in response to altered nutrition could be involved in the development or progression of diabetes in human.

Oxidative stress

Disturbances in the normal redox state of cells can cause direct toxic effects through the production of ROS (peroxides and free radicals) that damage all components of the cell, including DNA and histones (Fig. 2d). For example, 5′-oxidation of the DNA component deoxyribose results in the formation of reactive 3′-formylphosphate groups, which consequently induce the N6-formylation of histone lysine residues [159]. Lysine formylation is a widespread histone modification found both in core nucleosomes, at residues frequently acetylated or methylated, as well as in linker histones [159], The frequency of formylation in histones can increase several-fold upon oxidative stress and interfere with the signaling functions of lysine acetylation and methylation and thus contribute to the pathophysiology of oxidative stress [159]. Enhanced oxidative damage can also convert guanine into 8-oxoguanine, and 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), both of which reduce profoundly the binding of the methyl-binding protein MeCP2 to DNA [160]. Another methyl-binding protein, Mbd3, binds preferentially 5hmC relative to 5mC in vitro [161].

An increase in oxidative stress can also lead to epigenetic alterations by modifying the production of the coenzymes used by DNA and histone demethylases (Fig. 2d). Recently, it was found that the conversion of 5mC into 5hmC is mediated by the TET proteins (ten-eleven translocation), which

are αKG and Fe^{2+}-dependent enzymes [14]. This process is now considered an important pathway by which DNA demethylation occurs [162]. It has been proposed that under conditions of increased oxidative stress the citric acid cycle produces higher amounts of αKG, which then increases the activity of the TET enzymes [163]. Another group of demethylases that require αKG and Fe^{2+} for their activity are the Jumonji domain-containing histone demethylases which demethylate mono-, di-, and tri-methyl lysine residues and generate formaldehyde and succinate as byproducts [164]. In contrast, the histone demethylase LSD1/KDM1, which demethylates specifically H3K4me1/2 or H3K9me1/2 via an oxidation reaction that generates formaldehyde, requires FAD as a cofactor whose levels can also be altered by an increased oxidative stress [135, 164].

Another mechanism by which oxidative stress can induce epigenetic alterations consists of redistribution of specific chromatin modifying proteins across the genome (Fig. 2d). Under normal conditions, SIRT1 binds and represses repetitive DNA, as well as a well-defined, but functionally diverse set of genes spread across the genome [165]. In response to DNA damage induced by oxidative stress in vitro, SIRT1 dissociates from these loci and relocalizes to sites of DNA breaks to promote repair, resulting in transcriptional upregulation of those genes that become depleted in SIRT1 [165]. Notably, the redistribution of SIRT1 across the genome and the resulting transcriptional changes observed upon induction of oxidative stress in vitro parallel the in vivo effects of aging in the mouse brain. These observations suggest that oxidative stress-induced redistribution of SIRT1 and other chromatin modifying proteins may be a major mechanism that alters gene expression during aging [165].

All together, these results suggest that oxidative damage of DNA or histones and altered activities of DNA and histone demethylases induced by oxidative stress can have important effects on the epigenetic landscape and potentially result in heritable epigenetic changes in chromatin organization. This is particularly important in the context of the endocrine pancreas because it is particularly sensitive to oxidative stress (see section "Age-associated epigenetic alterations") and a wide variety of environmental cues have now been shown to lead to increases in the oxidative stress with functional consequences. Accordingly, rats exposed to a LP maternal diet in utero and during lactation exhibit age-associated increased oxidative stress and impairment of oxidative defense in their pancreatic islets [166, 167]. Early alterations in the expression of oxidative stress-related genes are also observed in pancreatic islets of mice exposed to high-fat diet [168, 169]. Importantly, reducing the oxidative stress by pharmacological or genetic means leads to improved β cell function in multiple mouse models of diabetes [170, 171].

Concluding remarks and perspectives

A number of recent studies have led to the identification of a network of transcription factors that control the development and the maintenance of differentiated states of the endocrine pancreas. It is now emerging that epigenetic mechanisms also play critical roles in these processes, with accumulating evidence for a crosstalk between the transcriptional and epigenetic developmental programmes. It is likely that future studies will elucidate the mechanisms by which such crosstalk occurs and unravel more connections between genetic and epigenetic processes. Understanding the epigenetic programme of the developing endocrine pancreas will require a comprehensive view of the epigenetic events that occur at each step of the pancreas development. High-resolution epigenome maps, obtained in pancreatic cells isolated at different stages of development, combined with new mouse models bearing pancreas-specific deletions of various epigenetic regulators, will undoubtedly provide important new insights. The integration of epigenome with SNP mapping in human islets will also further our understanding about epigenotype–genotype correlations. For example, SNPs affecting the binding of transcription factors that recruit chromatin-modifying factors and alter the epigenetic landscape such as DNAse I hypersensitive sites have already been reported [172]. It is plausible that a number of GWAS SNPS that are associated with type 2 diabetes mediate their effects through modulation of the epigenetic landscape.

The connection between cellular metabolism and epigenetics is a growing field of research that gained momentum with the recent discovery of links between Acetyl-CoA and NAD^+/NADH and histone acetylation. Increasing our knowledge on metabolic signaling to the chromatin will be instrumental in deciphering how environmental stimuli influence the epigenome at the cellular and organism levels, and will help to elucidate the effects of oxidative stress, endocrine disruptors, diet, etc. on normal pancreatic function.

The epigenomic basis of diabetes is a very appealing new mechanism for the disease but faces major challenges. Unlike genetics, the epigenome varies in a cell-type-dependent manner. This means that there is a need to flow sort or laser capture microdissect biological material in order to achieve cell purity. There are also other challenging issues relating to human sampling, e.g., limited accessibility to post-mortem tissues and biopsy of samples across the life-course, and difficulties in precisely matching cases and controls. From a mechanistic point of view, the epigenomic basis of human disease has been historically rooted on the concept of "epimutation", which is a relatively common finding in cancer but also described in imprinting disorders. In cancer, the epimutations and genetic mutations that drive tumorigenesis are selected for and are therefore highly

represented in tumors. However, "epimutations" contributing to diabetes will not be selected for and the frequency of these events will be "harder" to quantify. The classic silencing mechanism by DNA methylation that is observed in cancers at promoter regions of tumor suppressor genes, may also apply to key "diabetes" genes and T2D pathogenesis but this remains unproven. Recent genome-wide studies in islets of T2D patients give support for a less "dramatic" role of DNA methylation (small differences were described to affect a wide number of genes). The contribution of altered histone marking to the etiopathogenesis of T2D remains unexplored. Of particular interest is the H3K27me3 mark, which is connected to the transcriptional memory systems mediated by the Polycomb genes. This mark has been shown to be highly enriched in rodent pancreatic islets at the key diabetes gene *Hnf4a* in response to early sub-optimal nutrition and/or aging [61, 87]. The *Hnf4a* work raised the interesting possibility that epigenetic-deregulation may occur at high frequencies at regulatory cis-elements throughout the genome, such as enhancers, which may disturb interactions with promoters and thus lead to subtle effects on gene transcription [61]. These small and additive effects on global gene transcription may in turn contribute to increased risk of T2D. Future studies involving high-throughput methodology to characterize long-range chromatin interactions (5C—chromosome conformation capture carbon-copy, ChIA-PET—Chromatin Interaction Analysis with Paired-End-Tag sequencing) and nuclear organization (HiC—genome-wide chromosome conformation capture) will be instrumental in unraveling the mechanisms of epigenome control during pancreatic development and how these mechanisms are influenced by the environment. By creating interactome maps between enhancers, promoter regions and their regulatory proteins, we will be able to identify better unique targets for therapeutic intervention. Ultimately, a full understanding of the epigenetic processes that underlie the developmental and environmental programming of the endocrine pancreas has indeed the potential to facilitate the discovery of novel tools for the treatment of T2D.

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References

1. Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:4–14
2. Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. Lancet 365:1333–1346
3. Bloomgarden ZT (2004) Diabetes complications. Diabetes Care 27:1506–1514
4. Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H (1999) Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance—a population-based twin study. Diabetologia 42:139–145
5. Doria A, Patti ME, Kahn CR (2008) The emerging genetic architecture of type 2 diabetes. Cell Metab 8:186–200
6. McCarthy MI (2010) Genomics, type 2 diabetes, and obesity. N Engl J Med 363:2339–2350
7. Bonnefond A, Froguel P, Vaxillaire M (2010) The emerging genetics of type 2 diabetes. Trends Mol Med 16:407–416
8. Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414:782–787
9. Jin W, Patti ME (2009) Genetic determinants and molecular pathways in the pathogenesis of type 2 diabetes. Clin Sci (Lond) 116:99–111
10. Jirtle RL, Skinner MK (2007) Environmental epigenomics and disease susceptibility. Nat Rev Genet 8:253–262
11. Hochberg Z, Feil R, Constância M, Fraga M, Junien C et al (2010) Child health, developmental plasticity, and epigenetic programming. Endocr Rev 32:159–224
12. Bird A (2007) Perceptions of epigenetics. Nature 447:396–398
13. Suzuki MM, Bird A (2008) DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet 9: 465–476
14. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H et al (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324:930–935
15. Ito S, Shen L, Dai Q, Wu SC, Collins LB et al (2011) Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science 333:1300–1303
16. Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21:381–395
17. Talbert PB, Henikoff S (2010) Histone variants—ancient wrap artists of the epigenome. Nat Rev Mol Cell Biol 11:264–275
18. Bai L, Morozov AV (2010) Gene regulation by nucleosome positioning. Trends Genet 26:476–483
19. Ringrose L, Paro R (2004) Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet 38:413–443
20. Brenner C, Fuks F (2007) A methylation rendezvous: reader meets writers. Dev Cell 12:843–844
21. Simon JA, Kingston RE (2009) Mechanisms of Polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 10:697–708
22. Grewal SI (2010) RNAi-dependent formation of heterochromatin and its diverse functions. Curr Opin Genet Dev 20: 134–141
23. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A (2009) An operational definition of epigenetics. Genes Dev 23:781–783
24. Reik W (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447:425–432
25. Ozanne SE, Constância M (2007) Mechanisms of disease: the developmental origins of disease and the role of the epigenotype. Nat Clin Pract Endocrinol Metab 3:539–546
26. Gites GK (2009) Developmental biology of the pancreas: a comprehensive review. Dev Biol 326:4–35
27. Pan FC, Wright C (2011) Pancreas organogenesis: from bud to plexus to gland. Dev Dyn 240:530–565
28. Zaret KS, Grompe M (2008) Generation and regeneration of cells of the liver and pancreas. Science 322:1490–1494

29. Zaret KS (2008) Genetic programming of liver and pancreas progenitors: lessons for stem-cell differentiation. Nat Rev Genet 9:329–340
30. Puri S, Hebrok M (2010) Cellular plasticity within the pancreas—lessons learned from development. Dev Cell 18:342–356
31. Scaglia L, Cahill CJ, Finegood DT, Bonner-Weir S (1997) Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. Endocrinol 138:1736–1741
32. Bonner-Weir S, Li WC, Ouziel-Yahalom L, Guo L, Weir GC et al (2010) Beta-cell growth and regeneration: replication is only part of the story*
33. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ et al (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326
34. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E et al (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448:553–560
35. Xu CR, Cole PA, Meyers DJ, Kormish J, Dent S et al (2011) Chromatin "prepattern" and histone modifiers in a fate choice for liver and pancreas. Science 332:963–966
36. van Arensbergen J, García-Hurtado J, Moran I, Maestro MA, Xu X et al (2010) Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. Genome Res 20:722–732
37. Haumaitre C, Lenoir O, Scharfmann R (2008) Histone deacetylase inhibitors modify pancreatic cell fate determination and amplify endocrine progenitors. Mol Cell Biol 28:6373–6383
38. Lenoir O, Flosseau K, Ma FX, Blondeau B, Mai A et al (2011) Specific control of pancreatic endocrine β- and δ-cell mass by class IIa histone deacetylases HDAC4, HDAC5, and HDAC9. Diabetes 60:2861–2871
39. Rai K, Nadauld LD, Chidester S, Manos EJ, James SR et al (2006) Zebra fish Dnmt1 and Suv39h1 regulate organ-specific terminal differentiation during development. Mol Cell Biol 26:7077–7085
40. Anderson RM, Bosch JA, Goll MG, Hesselson D, Dong PD et al (2009) Loss of Dnmt1 catalytic activity reveals multiple roles for DNA methylation during pancreas development and regeneration. Dev Biol 334:213–223
41. Dhawan S, Georgia S, Tschen SI, Fan G, Bhushan A (2011) Pancreatic β cell identity is maintained by DNA methylation-mediated repression of Arx. Dev Cell 20:419–429
42. Papizan JB, Singer RA, Tschen SI, Dhawan S, Friel JM et al (2011) Nkx2.2 repressor complex regulates islet β-cell specification and prevents β-to-α-cell reprogramming. Genes Dev 25:2291–2305
43. Chen H, Gu X, Su IH, Bottino R, Contreras JL et al (2009) Polycomb protein Ezh2 regulates pancreatic beta-cell Ink4a/Arf expression and regeneration in diabetes mellitus. Genes Dev 23:975–985
44. Deering TG, Ogihara T, Trace AP, Maier B, Mirmira RG (2009) Methyltransferase Set7/9 maintains transcription and euchromatin structure at islet-enriched genes. Diabetes 58:185–193
45. Francis J, Chakrabarti SK, Garmey JC, Mirmira RG (2005) Pdx-1 links histone H3-Lys-4 methylation to RNA polymerase II elongation during activation of insulin transcription. J Biol Chem 280:36244–36253
46. Párrizas M, Maestro MA, Boj SF, Paniagua A, Casamitjana R et al (2001) Hepatic nuclear factor 1-alpha directs nucleosomal hyperacetylation to its tissue-specific transcriptional targets. Mol Cell Biol 21:3234–3243
47. Luco RF, Maestro MA, Sadoni N, Zink D, Ferrer J (2008) Targeted deficiency of the transcriptional activator Hnf1alpha alters subnuclear positioning of its genomic targets. PLoS Genet 4:e1000079
48. Kubicek S, Gilbert JC, Fomina-Yadlin D, Gitlin AD, Yuan Y et al (2012) Chromatin-targeting small molecules cause class-specific transcriptional changes in pancreatic endocrine cells. Proc Natl Acad Sci USA 109:5364–5369
49. Bhandare R, Schug J, Le Lay J, Fox A, Smirnova O et al (2010) Genome-wide analysis of histone modifications in human pancreatic islets. Genome Res 20:428–433
50. Stitzel ML, Sethupathy P, Pearson DS, Chines PS, Song L et al (2010) Global epigenomic analysis of primary human pancreatic islets provides insights into type 2 diabetes susceptibility loci. Cell Metab 12:443–455
51. Mutskov V, Felsenfeld G (2009) The human insulin gene is part of a large open chromatin domain specific for human islets. Proc Natl Acad Sci USA 106:17419–17424
52. Thorrez L, Laudadio I, Van Deun K, Quintens R, Hendrickx N et al (2011) Tissue-specific disallowance of housekeeping genes: the other face of cell differentiation. Genome Res 21:95–105
53. Gaulton KJ, Nammo T, Pasquali L, Simon JM, Giresi PG et al (2010) A map of open chromatin in human pancreatic islets. Nat Genet 42:255–259
54. Morán I, Akerman I, van de Bunt M, Xie R, Benazra M et al (2012) Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. Cell Metab 16:435–448
55. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW et al (2007) Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 39:311–318
56. Hoffman BG, Robertson G, Zavaglia B, Beach M, Cullum R et al (2010) Locus co-occupancy, nucleosome positioning, and H3K4me1 regulate the functionality of FOXA2-, HNF4A-, and PDX1-bound loci in islets and liver. Genome Res 20:1037–1051
57. Sekiya T, Muthurajan UM, Luger K, Tulin AV, Zaret KS (2009) Nucleosome-binding affinity as a primary determinant of the nuclear mobility of the pioneer transcription factor FoxA. Genes Dev 23:804–809
58. Thompson RF, Fazzari MJ, Niu H, Barzilai N, Simmons RA et al (2010) Experimental intrauterine growth restriction induces alterations in DNA methylation and gene expression in pancreatic islets of rats. J Biol Chem 285:15111–15118
59. Volkmar M, Dedeurwaerder S, Cunha DA, Ndlovu MN, Defrance M et al (2012) DNA methylation profiling identifies epigenetic dysregulation in pancreatic islets from type 2 diabetic patients. EMBO J 31:1405–1426
60. Xu Z, Wei G, Chepelev I, Zhao K, Felsenfeld G (2011) Mapping of INS promoter interactions reveals its role in long-range regulation of SYT8 transcription. Nat Struct Mol Biol 18:372–378
61. Sandovici I, Smith NH, Nitert MD, Ackers-Johnson M, Uribe-Lewis S et al (2011) Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. Proc Natl Acad Sci USA 108:5449–5454
62. Bar-Nur O, Russ HA, Efrat S, Benvenisty N (2011) Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet Beta cells. Cell Stem Cell 9:17–23
63. Yang L, Li S, Hatch H, Ahrens K, Cornelius JG (2002) In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc Natl Acad Sci USA 99:8078–8083
64. Kodama S, Kühtreiber W, Fujimura S, Dale EA, Faustman DL (2003) Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science 302:1223–1227
65. Jiang J, Au M, Lu K, Eshpeter A, Korbutt G et al (2007) Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem Cells 25:1940–1953

66. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R et al (2006) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292:1389–1394
67. Tayaramma T, Ma B, Rohde M, Mayer H (2006) Chromatin-remodeling factors allow differentiation of bone marrow cells into insulin-producing cells. Stem Cells 24:2858–2867
68. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455:627–632
69. Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S et al (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 138:449–462
70. Thorel F, Népote V, Avril I, Kohno K, Desgraz R et al (2010) Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 464:1149–1154
71. Yang YP, Thorel F, Boyer DF, Herrera PL, Wright CV (2011) Context-specific α- to-β cell reprogramming by forced Pdx1 expression. Genes Dev 25:1680–1685
72. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C et al (1991) Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019–1022
73. Gluckman PD, Hanson MA, Cooper C, Thornburg KL (2008) Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359:61–73
74. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C et al (1991) Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019–1022
75. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ et al (1998) Glucose tolerance in adults after prenatal exposure to famine. Lancet 351:173–177
76. Remacle C, Dumortier O, Bol V, Goosse K, Romanus P et al (2007) Intrauterine programming of the endocrine pancreas. Diabetes Obes Metab 9(Suppl 2):196–209
77. Park JH, Stoffers DA, Nicholls RD, Simmons RA (2008) Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest 118:2316–2324
78. Petry CJ, Dorling MW, Pawlak DB, Ozanne SE, Hales CN (2001) Diabetes in old male offspring of rat dams fed a reduced protein diet. Int J Exp Diabetes Res 2:139–143
79. Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA et al (2010) Chronic high-fat diet in fathers programs β cell dysfunction in female rat offspring. Nature 467:963–966
80. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC (2005) Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 135:1382–1386
81. Suter M, Bocock P, Showalter L, Hu M, Shope C (2011) Epigenomics: maternal high-fat diet exposure in utero disrupts peripheral circadian gene expression in nonhuman primates. FASEB J 25:714–726
82. Carone BR, Fauquier L, Habib N, Shea JM, Hart CE (2011) Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 143:1084–1096
83. Burdge GC, Lillycrop KA, Phillips ES, Slater-Jefferies JL, Jackson AA et al (2009) Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. J Nutr 139:1054–1060
84. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S et al (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854
85. Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmühl Y et al (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat Neurosci 12:1559–1566
86. Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300
87. Ozanne SE, Sandovici I, Constância M (2011) Maternal diet, aging and diabetes meet at a chromatin loop. Aging 3:548–554
88. Chang AM, Halter JB (2003) Aging and insulin secretion. Am J Physiol Endocrinol Metab 284:E7–E12
89. Møller N, Gormsen L, Fuglsang J, Gjedsted J (2003) Effects of ageing on insulin secretion and action. Horm Res 60:102–104
90. Iozzo P, Beck-Nielsen H, Laakso M, Smith U, Yki-Järvinen H et al (1999) Independent influence of age on basal insulin secretion in nondiabetic humans. European Group for the Study of Insulin Resistance. J Clin Endocrinol Metab 84:863–868
91. Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C et al (2003) Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. Diabetes 52:1738–1748
92. Muzumdar R, Ma X, Atzmon G, Vuguin P, Yang X et al (2004) Decrease in glucose-stimulated insulin secretion with aging is independent of insulin action. Diabetes 53:441–446
93. Maedler K, Schumann DM, Schulthess F, Oberholzer J, Bosco D et al (2006) Aging correlates with decreased beta-cell proliferative capacity and enhanced sensitivity to apoptosis: a potential role for Fas and pancreatic duodenal homeobox-1. Diabetes 55:2455–2462
94. Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. Cell 130:223–233
95. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A et al (2006) p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 443:453–457
96. Lee SH, Itkin-Ansari P, Levine F (2010) CENP-A, a protein required for chromosome segregation in mitosis, declines with age in islet but not exocrine cells. Aging 2:785–790
97. Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46: 1733–1742
98. Kuhlow D, Florian S, von Figura G, Weimer S, Schulz N et al (2010) Telomerase deficiency impairs glucose metabolism and insulin secretion. Aging 2:650–658
99. Tarry-Adkins JL, Chen JH, Smith NS, Jones RH, Cherif H et al (2009) Poor maternal nutrition followed by accelerated postnatal growth leads to telomere shortening and increased markers of cell senescence in rat islets. FASEB J 23:1521–1528
100. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F et al (2005) Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci USA 102:10604–10609
101. Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T et al (2008) Intra-individual change over time in DNA methylation with familial clustering. JAMA 299:2877–2883
102. Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR et al (2009) Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet 5:e1000602
103. Rakyan VK, Down TA, Maslau S, Andrew T, Yang TP et al (2010) Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res 20:434–439
104. Thompson RF, Atzmon G, Gheorghe C, Liang HQ, Lowes C et al (2010) Tissue-specific dysregulation of DNA methylation in aging. Aging Cell 9:506–518
105. Grönniger E, Weber B, Heil O, Peters N, Stäb F et al (2010) Aging and chronic sun exposure cause distinct epigenetic changes in human skin. PLoS Genet 6:e1000971
106. Bocker MT, Hellwig I, Breiling A, Eckstein V, Ho AD et al (2011) Genome-wide promoter DNA methylation dynamics of

human hematopoietic progenitor cells during differentiation and aging. Blood 117:e182–e189
107. Song CX, Szulwach KE, Fu Y, Dai Q, Yi C et al (2011) Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat Biotechnol 29:68–72
108. Szulwach KE, Li X, Li Y, Song CX, Wu H et al (2011) 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat Neurosci 14:1607–1616
109. Cheung I, Shulha HP, Jiang Y, Matevossian A, Wang J et al (2010) Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. Proc Natl Acad Sci USA 107:8824–8829
110. Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S et al (2010) Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 328:753–756
111. Krishnan V, Chow MZ, Wang Z, Zhang L, Liu B et al (2011) Histone H4 lysine 16 hypoacetylation is associated with defective DNA repair and premature senescence in Zmpste24-deficient mice. Proc Natl Acad Sci USA 108:12325–31230
112. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W et al (2005) Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 8:19–30
113. Narita M, Narita M, Krizhanovsky V, Nuñez S, Chicas A et al (2006) A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126:503–514
114. Funayama R, Saito M, Tanobe H, Ishikawa F (2006) Loss of linker histone H1 in cellular senescence. J Cell Biol 175:869–880
115. O'Sullivan RJ, Kubicek S, Schreiber SL, Karlseder J (2010) Reduced histone biosynthesis and chromatin changes arising from a damage signal at telomeres. Nat Struct Mol Biol 17:1218–1225
116. Chen H, Gu X, Liu Y, Wang J, Wirt SE et al (2011) PDGF signalling controls age-dependent proliferation in pancreatic β cells. Nature 478:349–355
117. Margueron R, Li G, Sarma K, Blais A, Zavadil J et al (2008) Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. Mol Cell 32:503–518
118. Rankin MM, Kushner JA (2010) Aging induces a distinct gene expression program in mouse islets. Islets 2:4–11
119. Kamiya M, Judson H, Okazaki Y, Kusakabe M, Muramatsu M et al (2000) The cell cycle control gene ZAC/PLAGL1 is imprinted—a strong candidate gene for transient neonatal diabetes. Hum Mol Genet 9:453–460
120. Mackay DJ, Coupe AM, Shield JP, Storr JN, Temple IK et al (2002) Relaxation of imprinted expression of ZAC and HYMAI in a patient with transient neonatal diabetes mellitus. Hum Genet 110:139–144
121. Gardner RJ, Mackay DJ, Mungall AJ, Polychronakos C, Siebert R et al (2000) An imprinted locus associated with transient neonatal diabetes mellitus. Hum Mol Genet 9:589–596
122. Arima T, Drewell RA, Arney KL, Inoue J, Makita Y (2001) A conserved imprinting control region at the HYMAI/ZAC domain is implicated in transient neonatal diabetes mellitus. Hum Mol Genet 10:1475–1483
123. Du X, Rousseau M, Ounissi-Benkalha H, Marchand L, Jetha A et al (2011) Differential expression pattern of ZAC in developing mouse and human pancreas. J Mol Histol 42:129–136
124. Ma D, Shield JP, Dean W, Leclerc I, Knauf C et al (2004) Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. J Clin Invest 114:339–348
125. Ling C, Del Guerra S, Lupi R, Rönn T, Granhall C et al (2008) Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 51:615–622
126. Yang BT, Dayeh TA, Kirkpatrick CL, Taneera J, Kumar R et al (2011) Insulin promoter DNA methylation correlates negatively with insulin gene expression and positively with HbA(1c) levels in human pancreatic islets. Diabetologia 54:360–367
127. Yang BT, Dayeh TA, Volkov PA, Kirkpatrick CL, Malmgren S et al (2012) Increased DNA methylation and decreased expression of PDX-1 in pancreatic islets from patients with type 2 diabetes. Mol Endocrinol 26:1203–1212
128. Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P et al (2009) Parental origin of sequence variants associated with complex diseases. Nature 462:868–874
129. Toperoff G, Aran D, Kark JD, Rosenberg M, Dubnikov T et al (2012) Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. Hum Mol Genet 21:371–383
130. Donohoe DR, Bultman SJ (2012) Metaboloepigenetics: Interrelationships between energy metabolism and epigenetic control of gene expression. J Cell Physiol. doi:10.1002/jcp.24054
131. Hanover JA, Krause MW, Love DC (2012) Post-translational modifications: Bittersweet memories: linking metabolism to epigenetics through *O*-GlcNAcylation. Nat Rev Mol Cell Biol 13:312–321
132. Niculescu MD, Zeisel SH (2002) Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. J Nutr 132:2333S–2335S
133. Turner BM (2009) Epigenetic responses to environmental change and their evolutionary implications. Philos Trans R Soc Lond B Biol Sci 364:3403–3418
134. Teperino R, Schoonjans K, Auwerx J (2010) Histone methyl transferases and demethylases; can they link metabolism and transcription? Cell Metab 12:321–327
135. Cyr AR, Domann FE (2011) The redox basis of epigenetic modifications: from mechanisms to functional consequences. Antioxid Redox Signal 15:551–589
136. Feil R, Fraga MF (2012) Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet 13:97–109
137. Smith GC, Konycheva G, Dziadek MA, Ravelich SR, Patel S et al (2011) Pre- and postnatal methyl deficiency in the rat differentially alters glucose homeostasis. J Nutrigenet Nutrigenomics 4:175–191
138. Sandovici I, Smith NH, Ozanne SE, Constância M (2008) The dynamic epigenome: the impact of the environment on epigenetic regulation of gene expression and developmental programming. In: Tost J (ed) Epigenetics. Caister Academic, Norfolk, pp 343–370
139. Khulan B, Cooper WN, Skinner BM, Bauer J, Owens S et al (2012) Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia. Hum Mol Genet 21:2086–2101
140. Yu J, Auwerx J (2009) The role of sirtuins in the control of metabolic homeostasis. Ann N Y Acad Sci 1173(Suppl 1):E10–E19
141. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR et al (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324:1076–1080
142. Grummt I, Ladurner AG (2008) A metabolic throttle regulates the epigenetic state of rDNA. Cell 133:577–580
143. Guarente L (2011) The logic linking protein acetylation and metabolism. Cell Metab 14:151–153
144. Hallows WC, Lee S, Denu JM (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc Natl Acad Sci USA 103:10230–10235
145. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324:654–657

146. Katada S, Imhof A, Sassone-Corsi P (2012) Connecting threads: epigenetics and metabolism. Cell 148:24–28
147. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM et al (2011) The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab 13:517–526
148. Liu HK, Green BD, Flatt PR, McClenaghan NH, McCluskey JT (2004) Effects of long-term exposure to nicotinamide and sodium butyrate on growth, viability, and the function of clonal insulin secreting cells. Endocr Res 30:61–68
149. Vetterli L, Brun T, Giovannoni L, Bosco D, Maechler P (2011) Resveratrol potentiates glucose-stimulated insulin secretion in INS-1E beta-cells and human islets through a SIRT1-dependent mechanism. J Biol Chem 286:6049–6060
150. Zhang S, Roche K, Nasheuer HP, Lowndes NF (2011) Modification of histones by sugar β-N-acetylglucosamine (GlcNAc) occurs on multiple residues, including histone H3 serine 10, and is cell cycle-regulated. J Biol Chem 286:37483–37495
151. Fujiki R, Hashiba W, Sekine H, Yokoyama A, Chikanishi T et al (2011) GlcNAcylation of histone H2B facilitates its monoubiquitination. Nature 480:557–560
152. Gambetta MC, Oktaba K, Müller J (2009) Essential role of the glycosyltransferase sxc/Ogt in Polycomb repression. Science 325:93–96
153. Fujiki R, Chikanishi T, Hashiba W, Ito H, Takada I et al (2009) GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. Nature 459:455–459
154. Hanover JA, Lai Z, Lee G, Lubas WA, Sato SM (1999) Elevated *O*-linked *N*-acetylglucosamine metabolism in pancreatic beta-cells. Arch Biochem Biophys 362:38–45
155. Liu K, Paterson AJ, Chin E, Kudlow JE (2000) Glucose stimulates protein modification by *O*-linked GlcNAc in pancreatic beta cells: linkage of *O*-linked GlcNAc to beta cell death. Proc Natl Acad Sci USA 97:2820–2825
156. Soesanto Y, Luo B, Parker G, Jones D, Cooksey RC et al (2011) Pleiotropic and age-dependent effects of decreased protein modification by *O*-linked *N*-acetylglucosamine on pancreatic β-cell function and vascularisation. J Biol Chem 286:26118–26126
157. Lehman DM, Fu DJ, Freeman AB, Hunt KJ, Leach RJ et al (2005) A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective *N*-acetyl-beta-D glucosaminidase is associated with type 2 diabetes in Mexican Americans. Diabetes 54:1214–1221
158. Kebede M, Ferdaoussi M, Mancini A, Alquier T, Kulkarni RN et al (2012) Glucose activates free fatty acid receptor 1 gene transcription via phosphatidylinositol-3-kinase-dependent *O*-GlcNAcylation of pancreas-duodenum homeobox-1. Proc Natl Acad Sci USA 109:2376–2381
159. Wisniewski JR, Zougman A, Mann M (2008) Nepsilon-formylation of lysine is a widespread post-translational modification of nuclear proteins occurring at residues involved in regulation of chromatin function. Nucleic Acids Res 36:570–577
160. Valinluck V, Tsai HH, Rogstad DK, Burdzy A, Bird A et al (2004) Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). Nucleic Acids Res 32:4100–4108
161. Yildirim O, Li R, Hung JH, Chen PB, Dong X et al (2011) Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. Cell 147:1498–1510
162. Bhutani N, Burns DM, Blau HM (2011) DNA demethylation dynamics. Cell 146:866–872
163. Chia N, Wang L, Lu X, Senut MC, Brenner C et al (2011) Hypothesis: environmental regulation of 5-hydroxymethylcytosine by oxidative stress. Epigenetics 6:853–856
164. Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705
165. Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J et al (2008) SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell 135:907–918
166. Theys N, Clippe A, Bouckenooghe T, Reusens B, Remacle C (2009) Early low protein diet aggravates unbalance between antioxidant enzymes leading to islet dysfunction. PLoS One 4:e6110
167. Tarry-Adkins JL, Chen JH, Jones RH, Smith NH, Ozanne SE (2010) Poor maternal nutrition leads to alterations in oxidative stress, antioxidant defense capacity, and markers of fibrosis in rat islets: potential underlying mechanisms for development of the diabetic phenotype in later life. FASEB J 24:2762–2771
168. Dreja T, Jovanovic Z, Rasche A, Kluge R, Herwig R et al (2010) Diet-induced gene expression of isolated pancreatic islets from a polygenic mouse model of the metabolic syndrome. Diabetologia 53:309–320
169. Valle MM, Graciano MF, Lopes de Oliveira ER, Camporez JP, Akamine EH et al (2011) Alterations of NADPH oxidase activity in rat pancreatic islets induced by a high-fat diet. Pancreas 40:390–395
170. Tanaka Y, Tran PO, Harmon J, Robertson RP (2002) A role for glutathione peroxidase in protecting pancreatic beta cells against oxidative stress in a model of glucose toxicity. Proc Natl Acad Sci USA 99:12363–12368
171. Song B, Scheuner D, Ron D, Pennathur S, Kaufman RJ (2008) Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. J Clin Invest 118:3378–3389
172. McDaniell R, Lee BK, Song L, Liu Z, Boyle AP et al (2010) Heritable individual-specific and allele-specific chromatin signatures in humans. Science 328:235–239