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Epigenetic modifications and long non-coding RNAs influence pancreas development and function

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Abstract

Insulin-producing β -cells within the pancreatic islet of Langerhans are responsible for maintaining glucose homeostasis; the loss or malfunction of β -cells results in diabetes mellitus. Recent advances in cell purification strategies and sequencing technologies as well as novel molecular tools have revealed that epigenetic modifications and long non-coding RNAs represent an integral part of the transcriptional mechanisms regulating pancreas development and β -cell function. Importantly, these findings have uncovered a new layer of gene regulation in the pancreas that can be exploited to enhance the restoration and/or repair of β -cells to treat diabetes.

Keywords

Pancreas; islet; β -cells; epigenetic; long noncoding RNAs

Pancreas development

The pancreas is a bifunctional organ with exocrine and endocrine tissues regulating digestive processes and glucose homeostasis, respectively. These functionally distinct tissues arise from a common pancreatic progenitor pool in two major waves of differentiation termed the primary and secondary transitions (Figure 1) [1]. The primary transition refers to the specification of pancreas progenitors within the foregut endoderm and requires the essential transcription factors pancreatic and duodenal homeobox 1 (Pdx1) and pancreatic transcription factor 1a (Ptf1a). The secondary transition refers to the stage where pancreatic progenitors expand, and external signals direct cells toward endocrine versus exocrine fates. A critical phase of pancreatic islet differentiation is the activation of the basic helix-loop-helix transcription factor Neurogenin 3 (*Neurog3*) within a subset of pancreatic progenitor cells to specify the endocrine precursor pool; deletion of *Neurog3* results in the absence of endocrine cell development [2]. In the adult pancreas, there are four endocrine cell types that constitute the mature islet, including insulin-producing β -cells, glucagon-producing α -cells, somatostatin-producing δ -cells and pancreatic polypeptide-producing PP

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cells. Cell fate determination of these four endocrine cell populations within the Neurog3+ cells depends on a number of additional transcription factors including Pdx1, Nkx2.2, Pax4, Pax6, Isl1, NeuroD1, Arx, and Nkx6.1 (reviewed in [3]). In this review we discuss recent advances in the characterization of epigenetic changes that take place in the specification of the pancreatic endocrine cells, with a special focus on β -cells (summarized in Figure 1). We also discuss how epigenetics can play a role in the etiology and treatment of pancreas-related diseases and speculate on the putative role of long non-coding RNAs in the maturation and function of β -cells. These studies are primarily based on genetic models of murine pancreas development (Table 1), isolated rodent and human islets, and *in vitro* differentiated embryonic stem (ES) cells.

Epigenetic modifications during pancreas development

Endoderm differentiation

Cellular differentiation requires the establishment and maintenance of tissue specific patterns of gene expression in response to extracellular signaling. As with many developing tissues, epigenetic modifications within endodermal cells are dynamically positioned or removed to regulate gene expression in response to developmental cues. In particular, the promoters of lineage-determining factors are often enriched for epigenetic marks of both active and repressive chromatin (H3K4me3 and H3K27me3, respectively) in a bivalent “poised” state [4], which allows for rapid activation during development. In support of this idea, genomic analyses of endoderm differentiated from hESCs *in vitro* demonstrates that bivalent promoters are resolved to activate gene expression through depletion of H3K27me3, or repress gene expression through *de novo* H3K27 methylation [5, 6]. Consistently, H3K27me3 generated by the Polycomb repressive complex 2 (PRC2, see glossary) is necessary for the repression of pluripotency factors in the differentiating hESCs and for the appropriate specification of endoderm lineages [7]. Furthermore, the histone H3K27me2/3 demethylases, KDM6A and KDM6B, are upregulated after endoderm induction in hESCs [8], whereas their knockdown in hESCs significantly dysregulates WNT signaling and reduces the efficiency of endoderm specification [5, 8]. *In vivo*, definitive endoderm specification relies on extracellular signaling of Nodal/ActivinA via Smad2/3 phosphorylation to activate lineage specific transcription factors (for review see [9]). Based on *in vitro* differentiation studies, it has been proposed that the resolution of bivalent marks may be due to an interaction between KDM6B and SMAD2/3 causing loss of the H3K27me3 repressive mark in SMAD target genes [6, 10]. Consistently, the promoter of *Eomes*, a master regulator of endoderm induction [11, 12], is demethylated through the recruitment of Kdm6b and Tbx3 to a regulatory region upstream of the *Eomes* transcriptional start site (TSS). This facilitates enhancer-promoter association and demethylation of H3K27me3 to promote *Eomes* expression [13].

In addition to dynamic changes in histone methylation states, a subset of active endodermal genes also has a distinct signature of histone modifications at their enhancers [14]. The most common modification is the deposition of H2A.Z (associated with gene activation), suggesting a mechanism by which the enhancers of lineage-determinant genes are primed to be responsive to endodermal transcription factors. Lineage determination can also be

specified by the action of pioneer factors, which modify the chromatin to allow access of other cell-fate specific transcription factors. Endoderm differentiation is known to be dependent on two pioneering transcription factors, Foxa2 and Gata4 (reviewed in [15]). Foxa2 and H2A.Z regulate nucleosome depletion and gene activation of endodermal genes [16], and Gata4 is known to facilitate the acetylation of H3K27 mediated by the histone acetyltransferase p300 [17]. This suggests a potential mechanism by which endoderm specification is initiated by pioneer factors.

These studies and others provide evidence that chromatin remodeling mediated by histone methyltransferases (HMTs), histone demethylases (HDMs) and other histone modifications (Box 1) play essential roles in endoderm specification. However, the timely regulation of chromatin modification, the binding of transcription factors and how these transcription factors are targeted to the appropriate chromatin domain remains elusive.

Primary Transition

Signaling from the adjacent mesoderm induces the lineage-specific transcriptional program that specifies the ventral and dorsal pancreas within two distinct regions of the foregut endoderm. Recently, several *in vivo* studies have identified some of the key epigenetic changes associated with ventral pancreas induction. Within the prospective ventral pancreas endoderm, early mesodermal signals are believed to induce a bipotential precursor population for pancreas and liver [18]. The default fate of this ventral foregut endoderm region is activation of the pancreatic program; however fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling from the cardiac mesoderm and septum transversum actively block the pancreatic program to promote liver induction. Within this prospective liver domain, enhancer of zeste homolog 2 (Ezh2), a component of PRC2, represses *Pdx1* expression to restrain ventral pancreas induction and allow for liver specification [19]. Moreover, a global reduction of p300-dependent histone acetylation, which is associated with gene activation, prevents liver specification and results in the expansion of pancreatic domain. This data suggests that the endoderm is pre-patterned to specify the ventral pancreatic lineage, and that liver specification is facilitated by epigenetic regulation. Interestingly, loss of *Ezh2* does not affect dorsal pancreatic bud evagination and growth, suggesting that specification of the dorsal pancreas is independent of Ezh2. Although much is known about the source and identity of the signaling pathways and regulatory factors involved in dorsal pancreas induction [3], relatively little is known about the corresponding epigenetic events. However, *in vitro* hES cell differentiation studies have demonstrated that commitment to the pancreatic lineage requires stage-specific derepression of PcG-mediated repression through removal of H3K27me3 from the regulatory regions of genes encoding essential pancreatic transcription factors [5].

Once the pancreatic domains have been specified, FGF10 signaling from the mesenchyme promotes the expansion of Pdx1+ pancreatic progenitors [20]. During mitosis, many transcription factors and epigenetic marks are depleted from the chromatin, but cells must retain some memory of cell fate that will be passed to the progeny. Reactivation of the chromatin in a cell-type specific manner has been shown to be maintained by several mechanisms (reviewed in [21]) that include: 1) retention of epigenetic marks, such as

H3K9me3 and H3K27me3 [22], recruitment of epigenetic modifiers, such as Ring1b [23], and placement of histone variants such as H2A.Z [24]; 2) the ability of pioneer transcription factor to bind mitotic chromatin, such as Foxa [25] and Gata factors [22], which could potentially explain the pancreatic bud arrest phenotype in the conditional ablation of *Gata* factors [26, 27]; and 3) by the methylation of DNA by DNMTs. In support of the importance of DNA methylation, pancreas development was greatly abrogated when *Dnmt1* was deleted from pancreatic progenitors [28]. In the *Dnmt1* mutant mice, pancreatic progenitor cells were arrested at G2/M and underwent apoptosis. Similar results have been reported in zebrafish [29]. These studies demonstrate that DNA methylation is essential for early pancreas development, however it is still not known whether this is due to dysregulation of key pancreatic transcription factors or general genome destabilization. The fact that the *Dnmt1* mutant phenotype can be partially rescued by reducing the dosage of the transformation-related protein 53 (*Trp53*) gene supports the model of global genome instability [28, 29].

Secondary transition: Endocrine specification

Beginning around embryonic day (e) e12.5 in mouse, the pancreatic epithelium undergoes a major wave of cell differentiation. The three lineages of the pancreas, endocrine, exocrine and ductal cells, are allocated in a Notch-dependent manner (Figure 1). First, Notch allows the expansion of the pancreatic progenitor domain by repressing precocious differentiation. Second, a dynamic Notch gradient determines the allocation of the different pancreatic lineages [30]. Studies from many different groups have begun to elucidate the complex signaling pathways involved in these processes (reviewed in [31, 32]). Although it has been shown that the Notch signaling pathway interacts with chromatin modifiers in other tissues and organisms [33], the mechanism by which Notch signaling modifies chromatin structure during pancreas development is unknown and warrants future studies.

Differentiation of endocrine cells requires the expression of the endocrine master regulator *Neurog3* by a mechanism that has been proposed to involve lateral inhibition of Notch [34]. Genome-wide ChIP-seq analysis of H3K27me3 in sorted *Neurog3*⁺ cells showed increased PRC2-mediated methylation compared to sorted pancreatic progenitors [35]. However, in endocrine cells differentiated from hESCs there appeared to be a global decrease of H3K27me3 [5]. These epigenetic differences between the *in vivo* and *in vitro* differentiation of endocrine cells could be due to the presence of different inductive signals in the two systems and/or the use of alternative mechanisms of gene repression. However, both *in vitro* and *in vivo*, H3K27me3 marks are lost from the promoters of endocrine-specific transcription factors, suggesting the importance of HMT and HDM in endocrine-specific gene regulation.

It has also been shown that *Ezh2* suppresses the normal extent of endocrine cell induction; conditional homozygous and heterozygous deletion of *Ezh2* in *Pdx1*⁺ pancreatic progenitors results in increased numbers of *Neurog3*⁺ cells without changes in proliferation [35]. This suggests that the elevated *Neurog3*⁺ cell numbers may be due to increased specification at the expense of other lineages, although this has not been directly tested. Interestingly, conditional ablation of *Ezh2* in neuronal progenitor cells also results in the upregulation of

Neurog3 and enhanced neuronal differentiation [36], suggesting that a common mechanism exists in neurons and endocrine cells to regulate cellular differentiation.

Endocrine cells

The fundamental role of pancreatic islet endocrine cells is to secrete hormones to maintain glucose homeostasis. The temporal regulation of *Neurog3* expression, together with a combination of endocrine specific transcription factors, determines the specification of the different endocrine cell types in the islet. Loss- and gain-of-function studies have shown that HDACs are required for the establishment of the appropriate ratio of β - and δ -cells. Mice lacking *Hdac5* and *Hdac9* showed an increased β -cell mass and mice lacking *Hdac4* and *Hdac9* contained more δ cells [37].

β -cell mass has also been shown to be dependent on the levels of *Ezh2*. When a single allele of *Ezh2* is lost, there is augmented β -cell mass and improved glucose clearance in adult mice. However, complete loss of *Ezh2* resulted in reduced β -cell mass in the adult and mild hyperglycemia. Similar outcomes were reported in mouse pancreatic explants and in pancreatic cells differentiated from hESCs when chemically treated with inhibitors of *Ezh2* [35]. These findings suggest that the *Ezh2*-mediated repression of endocrine cell fate is conserved between mouse and human and *Ezh2* levels could be manipulated *in vitro* to improve the yield of β -cells. Moreover, mutations in *Ezh2* did not appear to affect the other endocrine populations suggesting that this could be a β -cell specific mechanism.

Histone methylation and ubiquitination have also been shown to regulate β -cell proliferation through transcriptional regulation of the *Ink4a* locus [38, 39]. Two cell cycle inhibitors *Cdkn2a* and *Cdkn2b* are encoded within this locus, of which *Cdkn2a* has been shown to be associated with the decline of β -cell proliferation observed in adult β -cells [40]. *Ezh2* and *Bmi1*, members of the PRC2 and PRC1 complex, respectively, maintain *Cdkn2a* repression in young β -cells, but their expression is lost in adult β -cells, leading to a reduction of repressive epigenetic marks and transcriptional activation. Loss of *Ezh2* expression has been shown to be associated with the age-dependent reduction of extracellular signaling mediated by platelet-derived growth factor (Pdgf) due to decreased expression of the Pdgf receptor [41]. Accordingly, global genetic deletion of either *Ezh2* or *Bmi1* resulted in reduced β -cell proliferation, decreased β -cell mass and glucose intolerance [38, 39]. Furthermore, re-establishment of Pdgf-mediated signaling in mice and isolated human islets restore *Ezh2* expression and β -cell proliferation in adult islets [41]. However, mice with conditional deletion of either *Ezh2* or *Bmi1* do not display defects in pancreas development, although these mice were unable to recover from chemically-induced pancreatitis [42, 43]. The inconsistency between these studies may be explained by the different ages analyzed, strain background, and/or the subtle nature of the endocrine defects that were observed in the original studies. Another study exploring the role of PRC1 components in mice demonstrated that the loss of *Ring1b* in pancreatic progenitors did not result in upregulation of *Cdkn2a*, nor lead to a reduction of β -cell mass. However, deletion of *Ring1b* did result in the aberrant expression of neural genes and β -cell dysfunction as measured by defects in glucose clearance. Interestingly, deletion of *Ring1b* in β -cells using the RIP:Cre allele did not result in any observable phenotype, suggesting that once *Ring1b* repression is

established, it is maintained independently of *Ring1b*. Maintenance of repression was shown to be dependent on H3K9me3 [44]. Although no phenotypes were observed in adult *Ring1b* mutant mice, it would be interesting to further examine how these animals compensate in more demanding metabolic conditions, such as high fat diet or pregnancy. It is possible that similar defects in β -cell replication *in vivo* will be observed once the challenged β -cells are induced to proliferate. Alternatively, different components of PRC1 may be required for distinct aspects of β -cell maturation and function.

With aging, not only are the repressive marks mediated by PRC1 and PRC2 lost in β -cells, but there is also an accumulation of H3K4me3 marks at the *Ink4a* locus causing transcriptional activation of *Cdkn2a* [38]. This permissive chromatin status of the *Ink4a* locus appears to be reverted during β -cell regeneration in a mouse model of diabetes [38]. Restoration of *Ezh2* activity is sufficient to induce proliferation of β -cells in young mice; however, in adult mice, deletion of *Mll1*, a member of the Trithorax group (TrxG) complex, is also required [45]. Cumulatively, there is evidence to suggest that β -cell proliferation is regulated by epigenetic modifications in the *Ink4a* locus mediated by the PRC1, PRC2, and TrxG complexes. The reinforcement of *Cdkn2a* expression in β -cells, first by losing H3K27me3 and later by accumulating H3K4me3 over time could be a defense mechanism to prevent unusually elevated numbers of β -cells that, in a normal situation, could lead to fatal hypoglycemia. Interestingly, reversibility of this “braking mechanism” could open the door to novel treatments of diseases associated with β -cell deficits, such as diabetes.

DNA methylation in β -cells has also been shown to be essential for the maintenance of β -cell identity. Conditional deletion of *Dnmt1* in immature β -cells results in ectopic expression of *Arx* and the cell fate conversion of β -cells into α -cells, causing glucose intolerance [47]. Furthermore, it has been shown that the homeobox factor *Nkx2.2* recruits a repressive complex that includes Hdac1 and Dnmt3a specifically to the *Arx* promoter in β -cells; disruption of this complex resulted in ectopic *Arx* expression in β -cells, leading to β - to α -cell conversion and diabetes [48].

Thus far we have discussed how epigenetic changes during pancreas development modify gene expression at discrete loci, however significant advances in sequencing technologies and computational approaches have allowed large-scale integrative analyses of gene expression, chromatin modifications and DNA methylation (Table 2) to generate a global snapshot of the human β -cell epigenome and transcriptome [49–52]. Maps of open chromatin in human islets reveal the existence of regulatory regions at TSSs, as expected, but have also revealed the presence of open chromatin in intergenic regions. Notably, the open chromatin at intergenic regions appears to be more islet specific than at TSSs, suggesting that these distal regulatory regions may confer tissue specific gene expression patterns [50, 51, 53]. These cis-regulatory regions are associated in clusters of open regulatory elements, termed COREs [50], some of which have enhancer characteristics (based on histone modifications) and are enriched for β -cell-related transcription factor sequence recognition motifs [53, 54]. These findings implicate a subset of transcriptionally active chromatin domains in the regulation of gene expression, as is seen in other cell types [55, 56]; but this has not been formally tested in islets. However, many of the SNPs associated with type 2 diabetes map to open chromatin regions outside of coding genes and

promoters [51–53, 57], suggesting that these regions are important for β -cell function (Box 2). Genome-wide profiles of histone modifications in hESC-derived β -cells also suggest that inappropriate chromatin remodeling during the *in vitro* differentiation process may contribute to the impaired metabolic functions associated with these β -like cells [5].

Dedifferentiation and trans-differentiation

Recently, two novel concepts related to cell plasticity, dedifferentiation and transdifferentiation, have influenced the β -cell biology field. The genetic or environmental origins of these two processes are represented in Figure 2. Dedifferentiation has been described in several genetic mouse models, including conditional deletion of von Hippel-Lindau (*Vhl*) [58] and *Foxo* genes [59], and the genetic reactivation of *Shh* signaling in β -cells [60]. In these conditions, β -cells appear to revert to a more dedifferentiated progenitor program of gene expression. Based on these findings and complementary data from mouse models of type 2 diabetes, it has been proposed that dedifferentiation may be a mechanism employed by the β -cell to elude cell death in conditions of glycemic stress. Furthermore, there is speculation that the dedifferentiated state is also associated with a more plastic epigenetic map, which would explain the increased cell fate changes observed in diabetic or stressed β -cells [59]. If this is true, epigenetic marks may represent new targets for treating type 2 diabetes. Furthermore, β -cells in a dedifferentiated state may be more receptive to induced proliferation that could restore euglycemia in diabetic patients.

Transdifferentiation has also emerged as a novel mechanism to generate new β -cells. Several studies have demonstrated the ability to reprogram cells within pancreas, intestine, liver and other tissues through the mis-expression of pancreatic transcription factors and/or modification of epigenetic marks. For example, overexpression of *MafA*, *Pdx1* and *Neurog3* in exocrine cells [61], *Pdx1* overexpression in liver cells [62], and *Pax4* overexpression in α -cells [63] converts the respective cell types into β -like cells. Cell fate conversion of α - to β -cells *in vitro* has also been demonstrated by inhibiting HMTs in isolated human islets [64]. Moreover, near complete ablation of β -cells with diphtheria toxin induces transdifferentiation of α - and δ -cells into β -cells, suggesting that circulating factors or paracrine signals can instruct the differentiation [65, 66]. Cell fate conversions have mostly been demonstrated between cell types that share developmental programs and common epigenetic characteristics [64, 67]. Interestingly, most of the trans-differentiation events occurred only in subpopulations of cells, suggesting that not every cell is responsive to the conversion stimuli and that some cells may retain a more permissive epigenetic state. Similar arguments are being considered to explain the low efficiency of iPS generation with the Yamanaka factors [68].

Long non-coding RNAs and pancreas development

Over the last decade, genome-wide analyses have revealed that most of the human genome is transcribed, including a large number of long non-coding RNAs (lncRNAs) longer than 200 nucleotides and with no protein-coding potential [69–71]. It is important to note that lncRNAs represents a broad definition of ncRNAs and includes several classes of functional RNAs, including enhancer RNAs (eRNAs) and intergenic-, sense- and antisense- lncRNAs. Although it is not clear what fraction of the currently annotated lncRNAs are functional,

there is increasing evidence of the involvement of lncRNAs in cell biology and human disease (see [72, 73] for review). Among the diverse cellular functions associated with lncRNAs, a recurrent theme is their ability to interact and recruit chromatin modifiers including histone modifiers and DNMTs [74]. However, despite the identification of increased numbers of lncRNAs, there is still very little evidence of their function in pancreas development and/or β -cell function [75, 76]. One reason that could account for this lack of functional information is the fact that lncRNA expression is extremely tissue- and stage-specific. Thus, their functional characterization will require tissue-specific analysis at different stages of development. Several recent reports have uncovered the existence of lncRNAs in the pancreatic islet. An integrative analysis of epigenetic modifications and gene expression identified many lncRNAs enriched in the islet of Langerhans [49]. This study showed that islet-enriched lncRNAs were conserved in mouse and human and developmentally regulated. Furthermore, it demonstrated that some lncRNAs were dysregulated in islets from type 2 diabetic patients. Another group reached similar conclusions by performing transcriptome reconstruction of isolated β -cells and mouse islets [77], and another study identified lncRNAs specific for β - and α -cells in human islets [64]. The potential importance of these islet-specific lncRNAs has been supported by genome-wide association studies (GWAS) to identify genetic variants underlying susceptibility to type 2 diabetes and metabolic disorders [78, 79]. Interestingly, although a few SNP candidates map to coding genes, a large proportion of the SNPs fall into non-coding regions, some of which are close to or within islet-specific lncRNAs [49] and cis-regulatory elements [53]. Overall, these studies suggest that lncRNAs may be part of the regulatory network of gene expression that regulates β -cell development and function; however, detailed functional analysis will be needed to begin to understand the biology of these novel non-coding genes.

Several of the known functions of lncRNAs may also be relevant to pancreas biology. For example, mammalian chromatin modifiers lack site-specific DNA response elements, and targeting of these complexes has been shown to be mediated by lncRNAs. Consistent with this, recent reports demonstrate that PRC2 can be targeted to nascent transcripts independent of the nucleotide sequence [80, 81]. Given that some enhancers can produce eRNAs, it is possible that histone modifiers are recruited to islet-specific eRNAs to regulate β -cell maturation and function. In addition, lncRNAs might play a role in the recruitment of cohesins and the stabilization of enhancer-promoter interactions [82–84]. Moreover, clusters of open chromatin elements and chromatin looping have been described in islets, and it is possible that lncRNAs are also involved in this process [85]. These are just some examples among many of how lncRNAs may regulate islet specific gene expression [86]. Overall, lncRNAs have been shown to be an integral part of gene regulation in many cellular systems, but their role in pancreas development and β -cell function has just recently begun to be appreciated. It is likely that in the near future, novel lncRNAs will be functionally characterized to further elucidate their regulation of pancreas development and β -cell function.

Concluding Remarks

Past studies of pancreas development and β -cell function have largely focused on coding genes. In recent years, great advances in cell purification strategies, *in vitro* differentiation

of β -cells from hESCs, and sequencing technologies have provided the scientific community with large datasets of islet-specific transcription factor binding sites, epigenetic modifications and gene expression profiles during different time points of mouse and human pancreas development (summarized in Table 2). The integrated analyses of these datasets have uncovered the existence of chromatin domains and lncRNAs with mostly unknown functions, but with potential major implications for β -cell development and/or function (Box2). Our current knowledge of pancreas development, the numerous genetic tools available [87] and the capacity to reproduce pancreas development *in vitro* [88, 89] warrant future studies exploring how chromatin structure, discrete chromatin transcriptional domains, and lncRNAs can be related to β -cell fate specification and function.

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Glossary

DNMTs	DNA methyltransferases. Catalyze the methylation of DNA, which, in mammals, occurs mainly on cytosines in CpG dinucleotides.
EZH2	Enhancer of zeste 2. A histone methyltransferase member of the polycomb repressive complex 2 that mediates the methylation of histone 3 on lysine 4 (H3K4me3); associated with transcriptional repression.
HATs	Histone acetyltransferases. Acetylate lysine residues of histones. Neutralize the negative charge of DNA relaxing the chromatin structure and promoting transcription.
HDACs	Histone deacetylases. Remove acetyl groups from lysine residues on histones, enabling chromatin to compact and causing transcriptional repression.
HDMs	Histone demethylases. Remove methyl groups from histones.
HMTs	Histone methyltransferases. Add methyl groups to lysine or arginine residues of histones.
KDM6	Family of histone demethylases that includes KDM6a and KDM6b. Remove methyl groups from lysine 27 di- and tri-methylated on histone 3 (H3K27me2/3)
PRC2	Polycomb repressive complex 2. Complex of proteins with histone methyltransferase activity. Primarily trimethylates histone 3 on lysine K27 (H3K27me3) and represses transcription.
PRC1	Polycomb repressive complex 1. Monoubiquitylates histone H2A on lysine 119 (H2AK119ub1) and represses transcription.

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Box 1. Epigenetic modifications and development

Cellular differentiation requires the establishment and maintenance of tissue specific patterns of gene expression in response to extracellular signaling to generate the variety of cell types present in an organism. According to the classic model of Waddington (reviewed in [90, 91]) this process is accompanied by epigenetic modifications that shape the structure of the chromatin, restrict differentiation potential and confer cellular specialization [92–94]. In agreement with this model, reprogramming of somatic cells required the depletion of epigenetic marks [95]. In addition, differentiation of ES or induced pluripotent stem (iPS) cells required epigenetic factors [96]. Cumulatively, this indicates the importance of epigenetics in cell fate specification.

Epigenetic modifications are dynamically positioned or removed in response to different developmental cues. The most common types of modifications include 1) methylation of DNA by DNA methyl transferases (DNMTs); 2) post-translational modification of histones, including acetylation (histone acetyltransferases (HATs) and deacetylases (HDACs), histone methylation (histone methyltransferases (HMT) and demethylases (HDM)), and histone ubiquitination (histone ubiquitin ligases); and 3) histone remodeling with the incorporation of histones variants such as H2A.Z and H3.3. ([97, 98].

Box 2. Type 2 diabetes, epigenetics and long non coding RNAs

Type 2 diabetes (T2D) results from the loss of glucose homeostasis due to insulin resistance and/or beta cell dysfunction associated with genetic factors, lifestyle and aging. Genome wide association studies have identified over 70 loci that are robustly associated with T2D risk [99]; however, this can only explain about 10–15% of T2D cases. Interestingly, most of the causal SNPs fall outside of coding genes, suggesting that epigenetics and non-coding sequences, including regulatory regions and ncRNAs, are important for the pathophysiology of T2D. In support of this idea, studies in human populations affected by periods of famine showed that children born under starvation conditions were at higher risk of poor metabolic control [100] and T2D [101] later in life, suggesting a role for heritable factors that are independent of DNA sequence. Additional studies showed defects in DNA methylation at the IGF2 locus [102], however these changes were measured in blood cells and do not directly address the presence of epigenetic modifications specific to the islet. Animal models of prenatal under-nutrition showed that rats exposed to intrauterine growth retardation (IUGR) develop T2D in the adulthood [103] and this phenomenon correlated with misexpression of the essential islet transcription factor *Pdx1* as a result of epigenetic modifications in its promoter [104], similar to what it is observed in islets from T2D patients [105]. Also, progeny from rats fed a low protein diet during pregnancy and lactation showed epigenetic modifications at the *Hnf4a* locus in islet-derived DNA that prevents enhancer-promoter interaction and increases the risk of T2D [106]. Moreover, mice exposed to high fat diet showed differences in islet DNA methylation in loci associated with T2D [107]. Isolated human islets exposed to long chain saturated fatty acids also showed global changes in DNA methylation and gene expression, including in some genes which were dysregulated in islets from T2D subjects [108]. These examples suggest that the environment can affect gene expression through epigenetics, and could represent potential targets for drug therapy to prevent or revert diabetic pathologies. Recently, the scientific community has begun to perform integrative analysis of allelic variance traits associated with T2D using global DNA methylation and gene expression analysis from islets isolated from T2D and non-diabetic donors [109–112]. With this approach, several candidate loci have been identified in pathways related to beta cell function and T2D to warrant future detailed functional studies.

The role of lncRNAs in T2D has just begun to be appreciated, as discussed in the main text, but the potential implications are exciting. As proof of principle, it has been recently shown that *MEG3*, a lncRNA that contains multiple microRNAs, is hypermethylated and downregulated in islets from T2D donors. Interestingly, some of the mRNAs targeted by these *MEG3* microRNAs are aberrantly overexpressed in T2D suggesting that *MEG3* may be relevant in regulating beta cell function [113]. Furthermore, the lncRNA *H1-LNC25* was identified in isolated islets and shown to regulate the expression of *GLIS3*, a transcription factor that contains T2D risk variants [49].

Although, we are still in the early days of understanding how epigenetics and lncRNAs modulate the function of β -cells, there is much evidence to suggest that chromatin

modifications, chromatin structure and ncRNAs could be an integral part of gene regulation with the potential to be targets of novel therapies designed to treat T2D.

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Highlights

- Epigenetic modifications are required for the appropriate specification β -cells
- A global epigenome snapshot of the islet has been generated
- Islet-specific long non-coding RNAs have been identified
- Table compiling available Chip-Seq and RNA-seq datasets in the pancreas

Figure 1. Epigenetics and pancreas development

Schematic representation of pancreas development with epigenetic modifiers involved in the process depicted in boxes. (ES) embryonic stem cell, (E) Endoderm, (FE) Foregut endoderm, (Lv) Liver, (PP) pancreas progenitors, (EP) Endocrine progenitors, (Exo) Exocrine cell.

Figure 2. De-differentiation and trans-differentiation of β -cells

Recent examples of environmental and genetic factors that cause β -cell dedifferentiation and transdifferentiation.

Table 1

Histone and DNA modifiers involved in pancreas development and phenotypes

Modifier	Genotype	Experimental conditions	Phenotype	Ref.
Polycomb repressive complex 2				
Ezh2	<i>FoxA3^{Cre}; Ezh2^{fl/fl}</i>	Conditional deletion in foregut endoderm	Ventral pancreatic bud expansion	[19]
	<i>Pdx1^{Cre}; Ezh2^{fl/+}</i>	Conditional heterozygous in pancreas progenitors	Increased number of endocrine progenitors and β -cells	[35]
	<i>Pdx1^{Cre}; Ezh2^{fl/fl}</i>	Conditional homozygous deletion in pancreas progenitors	Increased β -cells at birth; β -cell proliferation defects	[35]
	<i>p48^{Cre}; Ezh2^{fl/fl}</i>	Conditional deletion in pancreas progenitors	Pancreas regeneration defects after cerulein-induced pancreatitis	[43]
	<i>RIP^{Cre}; Ezh2^{fl/fl}</i>	Conditional deletion in insulin producing cells	Decline in β -cell proliferation	[39]
	<i>RIP^{rtTA}; Ezh2^{OE}</i>	Conditional OE of <i>Ezh2</i> in insulin expressing cells	Combined with TrxG knockdown in isolated islets from old mice increased proliferation of β -cells.	[45]
Polycomb repressive complex 1				
Bmi1	<i>Bmi1^{-/-}</i>	Global knockout	No defects in pancreas development (assessed at 6w); defects in pancreas regeneration after pancreatitis	[42]
	<i>Bmi1^{-/-}</i>	Global knockout	Severe defects in β -cell proliferation; reduced β -cell mass at 10w	[38]
	<i>Bmi1^{+/-}</i>	Global heterozygote	Improved insulin sensitivity with reduced insulin secretion	[114]
Ring1b	<i>Pdx1^{Cre}; Ring1b^{fl/fl}</i>	Conditional homozygous deletion in pancreas progenitors	Impaired glucose tolerance	[44]
	<i>Rip^{Cre}; Ring1b^{fl/fl}</i>	Conditional deletion in insulin-producing cells	n/d	[44]
Histone acetyltransferases				
P300	<i>p300^{+/-}</i>	Global heterozygote	Expansion of ventral pancreatic bud	[19]
Gcn5l2	<i>FoxA3^{Cre}; Gcn5l2^{fl/fl}</i>	Conditional deletion in foregut endoderm	n/d	[19]
Class IIa Histone deacetylases				
Hdac4	<i>Hdac4^{-/-}</i>	Global knockout	Increased δ -cells in neonates	[37]
Hdac5	<i>Hdac5^{-/-}</i>	Global knockout	Increased δ - and β -cells at e18.5 and P7	[37]
Hdac9	<i>Hdac9^{-/-}</i>	Global knockout	Increased β -cells at e18.5 and P8	[37]
DNMTs				
DNMT1	<i>Pdx1^{Cre}; Dnmt1^{fl/fl}</i>	Conditional deletion in pancreas progenitors	Pancreatic agenesis	[28]
	<i>RIP^{Cre}; Dnmt1^{fl/fl}</i>	Conditional deletion in insulin producing cells	β - to α -cell conversion, glucose intolerance	[47]

Table 2

Datasets available for the study of pancreas development and β -cell function.

ChIP-Seq	ES/IPS	FE	PP	EP	β -cells	α -cells	e-cells	Islet	T2D	Exo	Accession numbers
H3K4me1			hES [57]					H [51–53]			(57) MTAB-1990 (51) GEOD-23784 (52) MTAB-189/191 (53) MTAB-1919
H3K4me2								H [52]			(52) MTAB-189/191
H3K4me3	M [67] hES[5]		M [67] hES[5]		H [64] M [67] hES[5]	H [64]		H [49, 51, 52, 115] M [67] hES[5]		H [64]	(67) MTAB-906/877 (5) MTAB-1086 (64) GEOD-50386 (49) MTAB-1294 (115) GEOD-51310/51311/51312
H3K36me3								H [49, 115]		M [67]	
H3K27me3	M [67] hES[5]	M [35] hES[5]	M [35, 67] hES[5]	M [35]	H [64] M [67] hES[5]	H [64]		H [52] M [54, 67] hES[5]		H [64] M [67]	(35) GEOD-56617 (54) GEOD-30298
H3K27ac								H [53, 115]			
H3K9me3								M [54]			
H3K79me2								H [51]			
H2A.Z								H [53]			
RNApolIII								H [49]			
CTCF								H [51, 53]			
Ring1b	M [44]							M [44]			(44) MTAB-1402
Islet TF			hES[57]					H [53, 116] M [54, 116]			(116) MTAB-1143
DNA methylation	H [117]							H [109–112, 117]	H [109–111]		(109) GEOD-21232 (110) journal access (111) GEOD-38642 (112) GEOD-62640 (117) GEOD-52238
RNA-seq	M [67]		M [67]		H [49, 64, 118] M [49, 67, 119]	H [64, 118] M [119]	M [120]	H [49, 115, 121] M [77]	H [121]	H [49, 64, 118]	(118) MTAB-463/465 (119) GEOD-54973 (121) GEOD-50398 (77) SRA056174
DNase I								H [51]			

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	ES/iPS	FE	PP	EP	β -cells	α -cells	ϵ -cells	Islet	T2D	Exo	Accession numbers
FAIRE								H[50]			

(ES) embryonic stem cell, (iPS) induced pluripotent stem cells, (FE) Foregut endoderm, (PP) pancreas progenitors, (EP) Endocrine progenitors, (T2D) Type 2 diabetic islets, (Exo) Exocrine cell, (hES) Human embryonic stem cells, (H) Human, (M) Mouse. Accession numbers to dataset repositories at the European Bioinformatic Institute or the DNA Data Bank of Japan are indicated with each corresponding reference and only referred to once.