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Maria L. Golson *Editor*

Molecular and Cell Biology of Pancreas Development, Function and Regeneration

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Editor

Molecular and Cell Biology of Pancreas Development, Function and Regeneration

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Preface

Diabetes poses an escalating global health threat, affecting over 500 million individuals worldwide. The common factor underlying all types of diabetes is insufficient insulin production, which hinders peripheral tissues from absorbing or sequestering glucose. Insulin, vital for glucose regulation, is produced by β cells housed within pancreatic islets. The normal development of the pancreas, its islets, and β cells is essential for maintaining blood glucose homeostasis and averting diabetes and its associated complications. In addition, abnormalities in the exocrine pancreas also lead to diseases. For example, anomalies in ductal differentiation result in truncated ducts, leading to conditions like chronic pancreatitis, recurrent acute pancreatitis, and pancreatic insufficiency.

Advancements in sequencing and imaging technologies, new computational methods facilitating the integration and analysis of vast datasets, and new preclinical models have accelerated research into pancreas formation and function. This book concentrates on recent breakthroughs in understanding pancreas development, differentiation, and cell-fate maintenance. Its intended audience includes researchers who possess a fundamental grasp of this field and aspire to keep informed on its developments. The chapter authors are active researchers in their topics.

The first and second chapters delve into intrinsic mechanisms governing pancreas development. The first chapter elucidates the influence of epigenetics on pancreas development and its impact on β-cell function. These areas have benefited from enhanced comprehension of the histone code and sequencing technology. The second chapter explores the formation of pancreatic structure in three-dimensional space, focusing on ductal development. Advancements in imaging techniques have largely facilitated progress in this area.

The next three chapters scrutinize the role of the mature β cell in health and disease, along with attempts to restore functional β-cell mass in the face of β-cell failure. The third chapter examines attempts to replenish β-cell mass in diabetes via endogenous β-cell replication or transdifferentiating other pancreatic cell types. This effort has been enabled by an understanding of transcription factor and signaling networks gleaned from mouse models and ex vivo studies of human pancreatic tissue. The fourth delves into the knowledge of β-cell heterogeneity in health and

disease, pushed by improvements in sorting live islet cells and single-cell technology. The fifth chapter discusses recent research regarding the amplifying pathways of insulin secretion, partly facilitated by breakthroughs in reporter molecules and imaging technology.

The sixth chapter delves into progress in δ-cell research, historically underexplored due to its rarity in human and mouse islets and a shared marker expression with neuronal and enteroendocrine cells. Advances in single-cell technologies, such as sequencing and imaging, and the development and implementation of unique mouse models and genetic reporters have accelerated research into this cell.

The last chapter describes the adverse effects of disrupted maternal metabolic health on β-cell development and function. Manipulations of preclinical models have driven research on this topic.

The work presented in these chapters demonstrates that new technologies have expanded the horizons of diabetes and pancreas research. Looking ahead, further technological advancement promises even greater progress in these fields. Continued investment in research and development, coupled with interdisciplinary collaboration, may bring breakthroughs in preventing, diagnosing, and treating diabetes and pancreatic disorders.

In closing, I want to thank the authors of this book and the publications referenced for their contributions to this body of work. Your dedication and efforts are vital for furthering pancreas and diabetes research.

Baltimore, MD Maria L. Golson

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Epigenetic Regulation of Pancreas Development and Function

Tanya Hans Pierre, Eliana Toren, Jessica Kepple, and Chad S. Hunter

Abstract The field of epigenetics broadly seeks to define heritable phenotypic modifications that occur within cells without changes to the underlying DNA sequence. These modifications allow for precise control and specificity of function between cell types—ultimately creating complex organ systems that all contain the same DNA but only have access to the genes and sequences necessary for their cell-type-specific functions. The pancreas is an organ that contains varied cellular compartments with functions ranging from highly regulated glucose-stimulated insulin secretion in the β-cell to the pancreatic ductal cells that form a tight epithelial lining for the delivery of digestive enzymes. With diabetes cases on the rise worldwide, understanding the epigenetic mechanisms driving β-cell identity, function, and even disease is particularly valuable. In this chapter, we will discuss the known epigenetic modifications in pancreatic islet cells, how they are deposited, and the environmental and metabolic contributions to epigenetic mechanisms. We will also explore how a deeper understanding of epigenetic effectors can be used as a tool for diabetes therapeutic strategies.

Keywords Islets · Epigenetics · Pancreas · Chromatin · Histone · Beta-cell · Diabetes

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Abbreviations

CTCF	CCCTC-binding factor
CBP	CREB-binding protein
DDR	DNA-damage response
DNMT	DNA methyltransferases
E	Embryonic day
HDT	Histone demethylases
HMT	Histone methyltransferases
JmjC	Jumonji domain-containing demethylases
MafA	MAF bZIP transcription factor A
MSI2	Musashi RNA binding protein 2
Paupar	Pax6 upstream antisense RNA
PRMT	Protein arginine methyltransferases
TET	ten-eleven translocation
TF	Transcription factor
T1D	Type 1 diabetes
ac	Acetylation
R	Arginine
BET	Bromodomains and Extra terminal domain
Cpa1	Carboxypeptidase-1
ceRNA	Competing endogenous RNA
DUBs	Deubiquitylases
DIO	Diet induced obesity
DSB	Double strand break
GWAS	Genome-wide association studies
GLP-1	Glucagon-like peptide 1
GSIS	Glucose-stimulated insulin secretion
HFD	High fat diet
H	Histone
HAT	Histone acetyltransferase
HDACs	Histone deacetylases
lncRNA	Long ncRNA
K	Lysine
MOF	Males absent on the first
MEG3	Maternally expressed gene 3
me	Methylation
miRISCs	miRNA-induced silencing complex
mRE	microRNA recognition element
Ngn3	Neurogenin-3
NAD	Nicotinamide adenine dinucleotide
ncRNA	Non-coding RNA
NOD	Nonobese diabetic
OGTT	Oral glucose tolerance test

Pdx1	Pancreatic duodenal homeobox 1
piRNA	piwi-interacting RNA
PRC	Polycomb repressive complex
ROIT	Regulator of insulin transcription
Rnf20	Ring finger 20
Rnf40	Ring finger 40
siRNA	Small interfering RNA
TSA	Trichostatin A
TrxG	Trithorax group
T2D	Type 2 diabetes
ub	Ubiquitination
Ucn3	Urocortin-3
5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
βFaar	β-cell function and apoptosis regulator
βLINC1	β-cell Long Intergenic Noncoding RNA 19

1 Introduction

Genomic DNA sequences provide the genetic code that governs cellular identity and dictates the biological processes required for life. While DNA nucleotide base-pair sequences contain genomic instructions, the fine-tuning of cellular diversity and function is achieved through epigenetics. Epigenetics defines heritable modifications of gene expression that do not directly alter underlying DNA sequences. A primary mechanism of epigenetic control is the regulation of chromatin accessibility to transcription factor (TF) and coregulator complexes, which can be influenced by histone and DNA modifications. Non-coding RNAs can also carry out epigenetic control through modification of chromatin structure by regulating the time and place in which genomic regions are accessible. Additionally, overall chromatin topography, not just accessibility, is also manipulated in epigenetics. The placement of enhancers, promoters, and other genetic regulatory elements dictates their ability to interact with genes and enable transcription. This intricate and dynamic regulatory system allows organs, such as the pancreas, to develop and function with numerous diverse compartments and cell types, despite having the same genetic code in every nucleus.

The varied cell types of the pancreas, each with unique functions, make it an ideal environment for delving into epigenetic control, especially during the highly regulated orchestration of embryonic development. Pancreatic development begins around embryonic day (E) 9.5 in mice, with dorsal and ventral pancreatic buds emerging on either side of the developing endodermal gut tube (Jorgensen et al. 2007). The first TF to demarcate multipotent pancreatic progenitors is pancreatic duodenal homeobox 1 (PDX1), and subsequent progenitors are classified into pools of either “tip” or “trunk” cells, as defined by Carboxypeptidase1 (CPA1) and Neurogenin-3 (Ngn3) expression, respectively (Zhou et al. 2007; Jonsson et al.

1994). While trunk cells will subsequently populate the exocrine compartment of the pancreas, the NGN3^{+} endocrine progenitors will differentiate into the five hormone-secreting cell types that populate the islet, including the insulin-producing β-cells, glucagon-producing α-cells, and somatostatin-producing δ-cells. Over time, PDX1 expression becomes enriched in β-cells, which require expression of the islet maturity TF marker MAFA (MAF bZIP transcription factor A), among others, for proper glucose sensing and insulin secretion (Zhang et al. 2005).

The primary responsibility of mature β-cells is to produce, package, and secrete insulin in response to glucose metabolism. In turn, insulin reduces blood glucose levels, protecting an organism from hyperglycemia. In addition to PDX1 and MAFA, the TFs NKX6.1, FOXA2, and NEUROD1 facilitate these functions of mature β-cells (Wortham and Sander 2021). Reduced expression of β-cell TFs or dysregulation of their transcriptional complexes, and thereby loss of β-cell function, disrupts glucose homeostasis and often results in the development of diabetes (Davidson et al. 2021). Type 2 diabetes (T2D) is characterized by the depletion of functional β-cell mass and peripheral insulin resistance and makes up the majority of global diabetes cases (Tinajero and Malik 2021). Briefly, during T2D, β-cell function intensifies to keep up with increasing glycemic demands from overnutrition or obesity. This increased demand can cause ER stress and unfolded protein response (UPR) in the β-cell. Chronic high blood glucose levels can have glucotoxic effects on the β-cell, which cause hypertrophy or hyperplasia that additionally contributes to dysregulation. Comprising only about 5% of diabetes cases, type 1 diabetes (T1D) results from autoimmune-mediated destruction of β-cells (Xie et al. 2020). Autoreactive T cells infiltrate the pancreas, activate inflammatory pathways, and subsequently trigger the destruction of insulin-producing β-cells. Both T1D and T2D are heritable diseases, but environmental factors that impact epigenetics and gene expression, including diet, the intrauterine environment, exercise, and viral exposure, can contribute to diabetes development and general β-cell dysfunction (Rewers and Ludvigsson 2016).

Ultimately, the combinatorial effect of TF actions with epigenetic effectors shape the diversity and specificity of pancreatic cell identity and function. In this chapter, we will define epigenetic modifications and their modifiers in the pancreas, identify environmental and metabolic contributions to epigenetic mechanisms, and explore how a deeper understanding of epigenetic effectors can be used as tools for various diabetes therapeutic strategies.

2 Types of Epigenetic Modifications and their Activities

2.1 DNA Modifications

2.1.1 Methylation and DNMTs

DNA methylation is a DNA modification that typically elicits gene silencing through the placement of a methyl group on a cytosine residue within a CpG dinucleotide. This addition changes the activity of the modified DNA segment without altering

the base-pair sequence itself. DNA methyltransferases (DNMTs) are responsible for depositing methyl groups, while TET enzymes facilitate their removal. TETs can reverse DNMT action through the conversion of the 5-methylcytosine (5mC) methylation mark into a 5-hydroxymethylcytosine (5hmC) intermediate that precedes thymine DNA glycosylase-mediated base-excision repair (Luo et al. 2018) (Fig. 1).

Of the four primary classes of DNMTs, DNMT1 is a key regulator of pancreas development and maintenance of α-cell and β-cell identity. Tandem loss of DNMT1 and an α-cell TF, ARX, in mature α-cells results in a transition to a β-cell-like state, highlighting potential plasticity of α-cells as well as DNMT1 importance (Chakravarthy et al. 2017). While DNMT1 loss triggers β-cell to α-cell reprogramming upon β-cell-specific loss, ablation of DNMT1 alone in α- or δ-cells has no effect on cell fate (Damond et al. 2017). Embryonic ablation of DNMT1 solely in α-cells, from conception to weaning, triggers a progressive reduction in adult α-cells and by 12-months DNMT1-deficient α-cell mice have fivefold less α-cells than controls. Developmental loss of DNMT1, however, did not impact α-cell maturity or differentiation. Similarly, deletion of DNMT1 in δ-cells had no effect on their differentiation or survival.

While DNA methylation patterns are established primarily during early development and tissue differentiation, they are frequently disrupted in disease states. Differential methylation patterns have been identified prior to and during the onset of both T1D and T2D, which adds therapeutic interest to investigating and understanding this epigenetic mark. Methylation has been suggested as a potential biomarker of T1D, where methylation near the *INSULIN* locus was observed in T1D

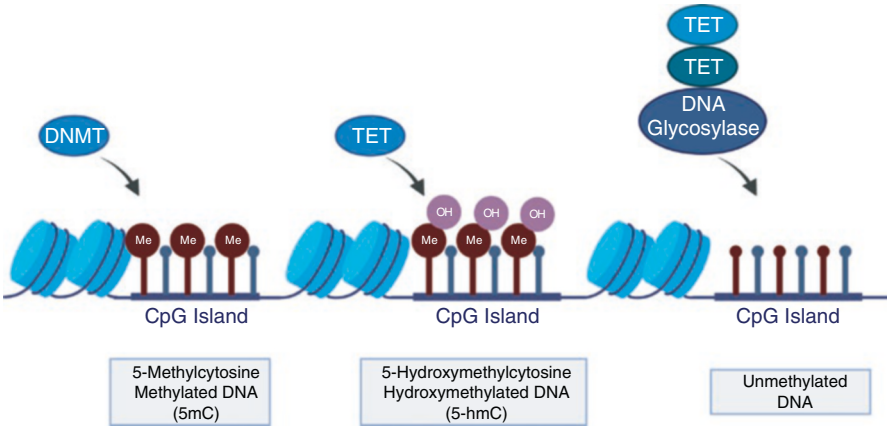

Fig. 1 DNMT and TET function. DNMT (DNA methyltransferase) enzymes place a methyl group (Me) on a cytosine (C) residue of a CpG dinucleotide in unmethylated DNA to create 5-methylcytosine (5mC) DNA. TET enzymes can reverse DNMT action by catalyzing the hydroxylation of 5mC into a 5-hydroxymethlcytosine (5hmC) via the addition of OH residues. This 5-hmC intermediate precedes base-excision repair by DNA glycosylases, which convert modified residues back to C, resulting in a return to the unmethylated state. Created with BioRender.com

patients (Carry et al. 2020). Interestingly, differentially methylated DNA, including *INSULIN*, may also be a biomarker of β-cell death (Liu et al. 2018). Longitudinal studies in T1D individuals showed differential methylation patterns in 10 genomic regions (primarily located near protein-coding genes) prior to islet-autoimmunity onset, suggesting that this pattern could be a precursor to T1D development (Johnson et al. 2020). As for T2D, hyperglycemia and high lipid exposure are associated with altered DNA methylation in pancreatic islets. Human islets incubated with high glucose demonstrated an overall increase in DNA methylation, including increased methylation of the *PDX1* distal promoter and enhancer regions (Hall et al. 2018). In a longitudinal study of individuals with T2D, peripheral blood samples were collected following an oral glucose tolerance test (OGTT), and T2D imparted increased differential methylation relative to control individuals throughout the study. Of the differentially methylated targets identified, *MSI2* (Musashi RNA binding protein 2) had a strong positive correlation with insulin sensitivity. MSI2 negatively regulates murine *insulin* expression, and its expression is responsive to T2D-like conditions of lipotoxicity and ER stress (Jeon et al. 2017).

2.2 Histone Modifications

Histones are the proteins around which eukaryotic DNA is tightly wrapped to allow extensive genomic material to fit within the nucleus. The DNA-histone complex forms chromatin, whose basic unit is the nucleosome (Fig. 2). Nucleosomes comprise an octamer of two of each of the histone proteins (histone(H) H2A, H2B, H3, H4) wrapped in 146 base pairs of DNA. The level of DNA compaction around histones can influence gene transcription, with more tightly wound DNA reducing accessibility of transcriptional machinery to genomic sequences. This compaction is controlled by post-translational amino- or carboxy-terminal histone tail modifications that influence downstream chromatin function (Fischle et al. 2003). The regulators that modify histone tails through the placement of modifications are called epigenetic writers. There are various types of epigenetic writers, including acetyltransferases, methyltransferases, ubiquitin ligases, and kinases. These epigenetic modifications are then recognized by readers, which are effector proteins that identify and interpret the histone marks left by each writer. Manipulation of these epigenetic modifications allows for multiple levels of cellular diversity, as marks encoded by writers can be dynamically reversed by their respective epigenetic erasers (Zhang et al. 2015). A list of these modifications and their general effects on transcription can be found in Table 1.

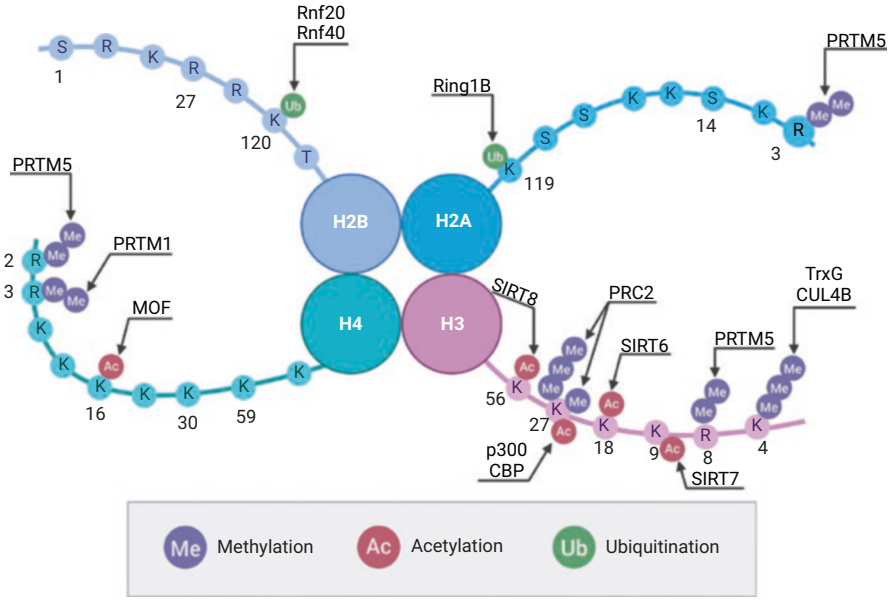

Fig. 2 Histones and their modifications. Histone octamer comprised two of each histone unit: H2A, H2B, H3, and H4, each with histone tails. These flexible histone tails can be post-translationally modified by various factors (black) that deposit methylation (purple), acetylation (red), or ubiquitination marks (green). These post-translational modifications (PTMs) affect DNA interactions by altering the charge on histone proteins and thereby tightly regulating nucleosome stability, gene accessibility, and gene expression. Created with BioRender.com

Table 1 Histone modifications and their general effects

Residue	Modifier	Transcriptional effect
Histone acetylation		
H3K27Ac	CBP and p300	Activation
H4K16Ac	MOF	Activation
H3K18Ac	SIRT6	Activation
H3K9Ac	SIRT7	Activation
H3K56Ac	SIRT8	Activation
Histone methylation		
H3K27me	PRC2	Activation
H3K27me3	PRC2	Repression
H3K4me3	TrxG and CUL4B	Activation
H4R3me2a	PRTM1	Activation
H2AR3me2s	PRTM5	Repression
H3R8me2s	PRTM5	Repression
H4R2me2s	PRTM5	Repression
Histone Ubiquitination		
H2AK119ub H2AK119ub	Ring1B Bmi1	Repression
H2BK120ub1	Rnf20 and Rnf40	Activation and repression

2.2.1 Histone Acetylation

HATs

Histone acetylation is a modification associated with open chromatin and, therefore, gene activation. Histone acetyltransferases (HATs) carry out acetylation by placing acetyl groups on the lysine (K) residues of histones. HATs can be classified into two main groups based on where they are localized within cells, with class A HATs localized to the nucleus and class B HATs to the cytoplasm (Gujral et al. 2020). Below, we detail various HATs in the pancreas and studies that have led to their discovery and characterization thus far.

CREB-binding protein (CBP) and p300 are structurally similar HATs that engage in H3K27Ac-mediated transcriptional co-activation and act as transcriptional scaffolds (He et al. 2021) (Fig. 2). CBP and p300 promote *insulin* and *Slc2a2* transcription via interactions with TFs NEUROD1, PDX1, and KLF11 (Zhang et al. 2021a). CBP and p300 also enhance β-cell gene expression through acetylation of the nucleosomes near cAMP-response element binding protein (CREB) target genes (Van de Velde et al. 2019). For instance, p300 regulates the expression of *TXNIP*, a key regulator of diabetic β-cell dysfunction, through an interaction with the ChREBP TF. Further investigations of the p300-TXNIP relationship in rat and human donor islets demonstrated that p300 expression levels increase in conditions of T2D, in parallel with TXNIP. Pharmacological and genetic inhibition of p300 reduces the expression of TXNIP under high glucose conditions, which increases insulin secretion and reduces β-cell apoptosis. These findings implicate p300 as a key mediator of TXNIP function and suggest that inhibition of its HAT activity may lessen TXNIP-mediated glucotoxicity and promote β-cell survival during diabetes (Bompada et al. 2016).

Conversely, p300 levels were reduced in β-cells and human islets exposed to T2D-like high glucose conditions (11–30 mM), islet amyloid polypeptide (IAPP), and inflammatory cytokines (Ruiz et al. 2018). This reduction was linked to glucolipotoxicity-induced proteasomal degradation of p300. Incongruent data on the role of p300 in diabetic conditions may reflect temporal differences in p300 regulation of pancreatic cells during development and disease.

In vivo analysis of *p300* deletion in developing endocrine progenitors, driven by *Ngn3-Cre*, revealed that mice did not develop glucose dysglycemia until after weaning (8 weeks old) (Zhang et al. 2021a). At this age, mice were glucose intolerant and had significantly reduced plasma insulin levels. Additionally, insulin content and β- and α-cell numbers were reduced relative to control mice. An islet-wide CBP deletion demonstrated a similar onset of glucose intolerance by 8 weeks of age, accompanied by reduced insulin content and β- and α-cell area. Reducing CBP expression in p300 knockouts (CBP^{Het};$p300^{KO}$) resulted in more severe glucose intolerance than either of the single gene knockouts ($p300^{KO}$ or CBP^{KO}) by 8 weeks of age; however, consistent reductions in insulin secretion, content, and β- and α-cell area were demonstrated. These reductions were also observed *in vitro* using a selective inhibitor of CBP/p300 HAT activity in rat islets (Wong et al. 2018).

Embryonic deletion of either p300 or CBP in endocrine progenitors reduced α-cell and β-cell area at E18.5, but the majority of tri-allelic knockout $CBP^{Het};p300^{KO}$ mice experience embryonic lethality or a complete lack of β- and α-cells (Wong et al. 2018). The observation of reduced β- and α-cell identity gene expression via RNA-seq of rat islets treated with this CBP/p300 inhibitor further supported this finding.

MOF (males absent on the first; also called MYST1 or KAT8) is a member of the MYST family (Moz, Ybf2/Sas2, Sas2, Tip60) of HATs and is the major enzyme that acetylates histone 4 at lysine 16 (H4K16). In the pancreas, MOF regulates DNA damage and α-cell differentiation (denDekker et al. 2020). Ablation of MOF expression in both lean and diet-induced obesity (DIO) mice decreased α-cell ratios (GCG^+/INS^+GCG^+). Fasting blood glucose levels and glucose tolerance were improved in MOF-deficient mice. These glucose homeostasis improvements were due to increased β-cell mass and enhanced glucagon-like peptide 1 (GLP-1) and insulin secretion (Guo et al. 2020). Furthermore, ChIP-Seq in an α-cell line treated with an MOF inhibitor, mg149, demonstrated that MOF regulates the expression of the TFs PAX6, NKX2.2, and FOXA2, essential for α-cell differentiation and function (Guo et al. 2021).

HDACs

Acetyl groups are removed by histone deacetylases (HDACs), which are also categorized into four classes based on their homology to yeast HDACs. Of these, class I and class II are the major HDACs involved in removing acetyl groups. HDACs are also grouped into two families based on whether they are zinc ion-dependent or nicotinamide adenine dinucleotide (NAD)-dependent. Class I HDACs are primarily localized within the nucleus and consist of the 1, 2, 3, and 8 isoforms. Class II HDACs are found in both the cytoplasm and nucleus and are categorized into IIa and IIb isoforms, depending on unique domain composition. Class IIa HDACs have an adaptor domain in the N-terminus and include isoforms 6 and 10. In contrast, class IIb HDACs have a tail domain at the C-terminus and include isoforms 4, 5, 7, and 9. Class III HDACs, also known as Sirtuin proteins, also localize to the cytoplasm and mitochondria. Class III HDACs are required for silencing transcription and are the only NAD^+-dependent family of HDACs. There is only one class IV HDAC, HDAC11, and it localizes to the nucleus and cytoplasm (Park and Kim 2020; Makkar et al. 2020).

CLASS I HDACs

All classes of HDACs are implicated in regulating β-cell function and metabolism. For instance, all 18 HDACs are expressed in β-cells, some class III HDACs are important for insulin secretion, class I HDACs regulate β-cell cytotoxicity, and class IIa HDACs modulate β- and δ-cell mass (Zhang et al. 2020a, 2019). HDAC1, a class I HDAC, has been extensively studied in the context of cell cycle progression in

cancer, although recent analyses have expanded on this role in β-cells. Adenoviral-mediated overexpression of NKX6.1 in rat islets prompted the expression of HDAC1, which is sufficient to induce β-cell proliferation. Overexpression of HDAC1 demonstrated that β-cell replication is induced by the reduction of the cell-cycle inhibitor Cdkn1b and the upregulation of cell-cycle activators (Draney et al. 2018).

Prolonged treatment of rat islets with the HDAC inhibitors trichostatin A (TSA) or sodium butyrate (SB) increased insulin secretion. Investigations of the HDAC imparting this improvement demonstrated that HDAC1 inhibition increases glucose-stimulated insulin secretion (GSIS), reduces *ad lib* blood glucose levels, and improves glucose tolerance in chow-fed mice by increasing Tph1-mediated serotonin synthesis (Zhang et al. 2020a). HDAC1 is also implicated in regulating insulin signaling by modulating *IRS2*. IRS2 is upregulated in mouse Min6 β-cells that have increased insulin secretion due to a higher passage number. Treatment of Min6 cells with broad HDAC inhibitors like TSA and the HDAC1-specific inhibitor apicidin recapitulates the increase in *IRS2*. However, treatment with HDAC1 inhibitor alone is unable to increase insulin secretion in Min6 cells (Kawada et al. 2017).

Deletion of *HDAC3* in adult mouse β-cells using a tamoxifen-inducible Cre driven by the mouse *insulin 1* promoter (*MIP-Cre*^{ERT}) similarly improves glucose tolerance and insulin secretion (Remsberg et al. 2017). Cistromic analyses revealed enrichment for HDAC3 binding near genes involved in endocrine cell development and the upregulation of GSIS targets, like *Rsg16* and *S100a6* (Calcyclin). Constitutive deletion of HDAC3 in mouse β-cells with a Cre recombinase driven by the rat *insulin 2* promoter (*RIP-Cre*) demonstrated contrasting results, with reduced insulin secretion and significant impairment of glucose tolerance upon HDAC3 loss (Chen et al. 2016). Aside from the limitations of differing Cre models, these varying results suggest HDAC3 may regulate β-cell identity and maintenance through distinct pathways.

CLASS II HDACs

Class II HDAC proteins, consisting of HDAC7, HDAC4, and HDAC6, also regulate insulin secretion. Islets from individuals with T2D display higher HDAC7 expression than islets from non-diabetics. Mitochondrial respiration is reduced in rat β-cells overexpressing HDAC7, indicating that dysregulation of mitochondrial function may contribute to T2D-associated insulin secretion and content reduction (Daneshpajooh et al. 2017). Studies of three individuals with T2D bearing missense mutations in *HDAC4* (H227R, D234N, and E374K), and recapitulation of these mutations in mouse β-cells, revealed impaired insulin secretion and reduced expression of the β-cell TFs PDX1, NEUROD1, and FOXO1 (Gong et al. 2019). Investigators found that HDAC4 mutations may impart these defects through disrupted FOXO1 nuclear translocation resulting from a lack of FOXO1 acetylation. Additionally, HDAC6 regulates insulin signaling, and its inhibition in mouse β-cells and islets using tubacin treatment resulted in the reduction of P-AKT and P-S6 signaling effectors. Furthermore, *Lepr*$^{db/db}$ mice, which have impaired insulin signaling

relative to lean mice, have reduced HDAC6 protein levels (Inoue et al. 2021). However, a study of class IIa HDACS in INS1E rat β-cells, cultured in normal and high glucose, suggests that HDAC4 may be dispensable for β-cell function; under- and overexpression of HDAC4 did not alter insulin expression, secretion, or β-cell viability (McCann et al. 2019).

CLASS III HDACs

Sirtuins are class III HDACs with NAD^+-dependent deacetylation activity. Of the seven sirtuins, SIRT1 and SIRT6 have been extensively studied and shown to regulate metabolism (Zhou et al. 2018). SIRT6 carries out deacetylation of H3K18Ac, H3K9Ac, H3K56Ac, and TFs like FOXO1 (Fig. 2). Reduction of SIRT6 expression in Min6 mouse β-cells impairs GSIS, with a similar dysregulation occurring in β-cell-specific and pancreas-wide SIRT6 knockout mice. Loss of SIRT6 in either β-cells or PDX1-expressing pancreatic cells results in glucose intolerance (Xiong et al. 2016a). This dysregulation of GSIS and glucose tolerance may result from disruption of glucose oxidation, mitochondrial damage, and disruption of calcium flux. Further studies of SIRT6 β-cell-specific knockouts suggest that GSIS dysregulation may be the result of disrupting the FOXO1 glucose sensing pathway (Song et al. 2016). FOXO1 negatively regulates PDX1, which promotes GLUT2 (*Slc2a2*) expression. In SIRT6 β-cell-specific knockout mice, FOXO1 is upregulated, and in turn, both PDX1 and GLUT2 are downregulated at the mRNA and protein levels. These findings, along with observations of SIRT6 deacetylation of FOXO1, suggest that SIRT6 regulates glucose sensing through interactions with PDX1 and GLUT2.

In addition to its roles in regulating GSIS, SIRT6 also protects β-cells against lipotoxicity. SIRT6 expression is reduced in islets of DIO mice, aged mice, and Min6 cells and mouse islets treated with palmitate (Xiong et al. 2016b). Overexpression of SIRT6, however, rescues Min6 mouse β-cells from palmitate-induced apoptosis. SIRT6 also protects β-cells by downregulating *TXNIP* expression (Qin et al. 2018). Other Sirtuins, including SIRT1–4, offer similar protection against β-cell apoptosis and insulin secretion aberrations. Overexpression of SIRT1–3 increases GSIS, while ablation of SIRT4 decreases GSIS (Zhang et al. 2016). In addition to the aforementioned β-cell roles, SIRT1 also regulates α-cell functional identity. Treatment of diabetic mice with a SIRT1 activator prevents diabetes-associated α-cell hyperplasia and glucagon increase, whereas treatment of the In-R1-G9 α-cell line with a SIRT1 activator significantly reduces glucagon secretion, α-cell proliferation, and *Arx* expression (Zhang et al. 2018).

Histone Acetylation Readers

Epigenetic readers must recognize acetylation marks on lysine tails of histones to modulate gene transcription. Bromodomain and extraterminal (BET) proteins specifically read acetylated lysines and enable acetylation-associated chromatin opening. While mainly studied in the context of cancer, recent studies have appreciated

roles for BET proteins in adipogenesis and pancreas development (Boyson et al. 2021; Lee et al. 2017). BET proteins, encoded by *Brd2*, *Brd3*, and *Brd4*, are expressed in mouse pancreatic buds beginning at E11.5 and are maintained in tissue explants up to E16.5. Pharmacological inhibition of BET proteins in mouse pancreatic bud explants and human iPSC (induced pluripotent stem cell) pancreatic progenitors increased *Ngn3*, *somatostatin*, *glucagon*, and *ghrelin* expression (Huijbregts et al. 2019). *MafA* and *Nkx6.1* increased following BET inhibition; however, insulin expression and content were only upregulated following the removal of chronic BET inhibition. *In vitro* inhibition of BET proteins potentiates endocrine cell marker expression, suggesting that these factors modulate endocrine cell development and differentiation.

2.2.2 Histone Methylation

Histone methylation silences or activates gene expression through the placement of methyl groups on arginine (R) and lysine (K) residues (Fig. 2). Methylation is carried out by histone methyltransferases (HMTs) and can be removed by histone demethylases (HDMs). The different methylation states—mono-, di-, tri-, or polymethylation—and their specific position determine whether gene transcription will be repressed or activated. For example, methylation of H3K79, H3K36, and H3K4 is generally associated with gene activation, whereas methylation of H4K20, H3K27, and H3K9 is associated with silencing (Hyun et al. 2017). Variability in histone methylation patterns facilitates complex regulation of pancreas development and islet cell differentiation.

Polycomb Group Proteins

Polycomb group proteins are transcriptional regulators that form epigenetic complexes, including the two most extensively studied Polycomb Repressive Complex 1 (PRC1) and PRC2 (Dumasia and Pethe 2020). In addition to recruiting PRC1 activity through methylation, PRC2 mono-, di-, and tri-methylates H3K27, which regulates endocrine pancreas and β-cell differentiation. EZH1 and EZH2 are the PRC2 methyltransferases that carry out this regulation. Additional complex components EED, SUZ12, and RBAP48 contribute to gene silencing and are essential for regulating endocrine pancreas specification, β-cell proliferation, and maintenance of β-cell identity (Lu et al. 2018; Dahlby et al. 2020). Most studies of the PRC2 methyltransferases focus on EZH2, primarily due to its higher methyltransferase activity and the fact that it is expressed in cells with greater rates of division (Golson 2021). EZH2 engages in transcriptional repression and activation, either alone or via interactions with polycomb components. Ablation of Raptor, an essential regulatory protein of mTORC1, in $NGN3^{+}$ progenitors demonstrated that EZH2 is involved in the G2/M phase and proliferation of neonatal β-cells (Wang et al. 2019). This observation is further supported by findings that β-cell-specific EZH2