CellPress

Focus on: The Endocrine Pancreas

Review

Chromatin Regulators in Pancreas Development and Diabetes

Stephanie A. Campbell[1] and Brad G. Hoffman[1,2,*]

The chromatin landscape of a cell is dynamic and can be altered by chromatin regulators that control nucleosome placement and DNA or histone modifications. Together with transcription factors, these complexes help dictate the transcriptional output of a cell and, thus, balance cell proliferation and differentiation while restricting tissue-specific gene expression. In this review, we describe current research on chromatin regulators and their roles in pancreas development and the maintenance of mature β cell function, which, once elucidated, will help us better understand how β cell differentiation occurs and is maintained. These studies have so far implicated proteins from several complexes that regulate DNA methylation, nucleosome remodeling, and histone acetylation and methylation that could become promising targets for diabetes therapy and stem cell differentiation.

Trends

The chromatin state of *cis*-regulatory loci is dictated by epigenetic modifications to DNA, histones, and nucleosomes.

Chromatin regulators modify chromatin to influence gene expression, development, and proliferation, which drive cellular transitions.

The processes of pancreas development and maintenance of mature, functional β cells depend on the actions of key chromatin regulators and transcription factors.

Proteins involved in chromatin regulation are in the spotlight as targets for future diabetes therapies and to improve stem cell differentiation.

Gene Regulatory Mechanisms Governing Pancreas Development and β Cell Maintenance

Diabetes mellitus is a complex disorder in which patients develop hyperglycemia due to an insufficient capacity of pancreatic β cells to secrete enough insulin to maintain glucose homeostasis. The two most common forms include type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). In T1DM, β cells are destroyed by the immune system, leading to dependence on exogenous insulin. In T2DM, hyperglycemia results from both insulin resistance in peripheral tissues and β cell dysfunction that leads to insufficient amounts of insulin being secreted in response to glucose. Although there has been significant progress in many areas of diabetes research, there is still no cure for the disease.

Transplantation of islets from cadaveric donors can provide insulin independence in >50% of patients [1]; however, the limited supply of pancreatic tissue reserves this treatment for the most brittle individuals. To overcome this shortfall, scientists have tried to generate pancreatic β cells both *in vitro* and *in vivo* by stimulating β cell proliferation [2], reprogramming other somatic cell types [3–6], and directing differentiation of human embryonic stem cells (hESCs) or iPSCs towards a β cell fate [7,8]. However, despite recent advances, the generation of cells that match the functional secretory ability of true β cells has not yet been achieved from hESCs *in vitro* due to our incomplete understanding of β cell development and differentiation. We, and others, believe that there is an underappreciated role for **chromatin structure** (see Glossary and Box 1) and epigenetic mechanisms that help dictate β cell specification and maturation [9,10]. During normal β cell genesis *in vivo*, the chromatin at critical ***cis*-regulatory loci** undergoes a series of alterations to allow the appropriate expression of transcription factor programs that define the different pancreas cell types and allow them

[1]Child and Family Research Institute, British Columbia Children's Hospital and Sunny Hill Health Centre, 950 W28th Avenue, Vancouver, BC, V5Z 4H4, Canada
[2]Department of Surgery, University of British Columbia, Vancouver, BC, V5Z 4E3, Canada

*Correspondence:
brad.hoffman@ubc.ca (B.G. Hoffman).

 http://dx.doi.org/10.1016/j.tem.2015.12.005

Box 1. Histone Modifications and Histone Variants Define Chromatin Structure

There are different possible chromatin states that are defined by the presence, absence, or combination of various histone modifications that occur at specific residues. For example, *cis*-regulatory loci can be found in several different possible states, including: (i) an active chromatin state, which is associated with the mono-, di- or trimethylation of histone 3 at lysine 4 (H3K4me1, me2, or me3) in combination with histone acetylation; (ii) a poised state, which is associated with the H3K4me1, me2, or me3 in the absence of histone acetylation; (iii) an inactive state, which is associated with a relative absence of histone modifications; (iv) a heterochromatin repressed state, which is associated with the trimethylation of histone 3 at lysine 9 (H3K9me3) and DNA methylation; (v) a Polycomb repressed state, which is associated with the trimethylation of histone 3 at lysine 27 (H3K27me3); or (vi) a bivalent state, which is associated with H3K4 methylation and H3K27me3 [86–88]. A diversity of other histone modifications tends to co-occur with these modifications and can help stabilize or modify the activity of the loci [86–88]. In addition, chromatin structure can be altered during histone turnover when the canonical nucleosome histone proteins (H2A, H2B, H3, and H4) are exchanged for histone variants, such as H2A.Z and H3.3 [89]. The modified chemical structure of histone variants affects the strength of the histone–DNA interaction and the ability for histone modifications to occur, thus controlling chromatin structure and gene expression. Regardless, the chromatin structure of the *cis*-regulatory loci of a cell can be dynamically altered to affect transcriptional changes, by the actions of various chromatin regulators that control nucleosome placement, DNA methylation, or the relative levels of the various histone modifications.

to become fully functional and mature [11,12]. Furthermore, it is clear that current *in vitro* protocols fail to completely allow these changes to occur [13], and only by understanding the changes that are normally required, and by defining the chromatin regulators responsible, will we be able to fully understand where and how the *in vitro* differentiation protocols are failing.

In this review, we outline the relation between epigenetics and gene regulatory mechanisms, summarize known chromatin modifications and associated complexes, and, finally, highlight current research on the roles that proteins in these complexes have in pancreas development and the maintenance of functional β cell maturity.

Pancreas Development: From Progenitors to Mature β Cells

The embryonic pancreas is first evident from the developing foregut endoderm as an epithelial bud that expresses the critical transcription factor Pdx1 at around embryonic day 9 (E9) in the mouse [14], which is induced by the Foxa1/Foxa2 transcription factors [15]. The resulting $Pdx1^{+}Sox9^{+}Ptf1a^{+}$ progenitors [16–18] subsequently give rise to all three pancreatic lineages: (i) the endocrine lineage, including insulin-secreting β cells, glucagon-secreting ∝ cells, somatostatin-secreting δ cells, ghrelin-secreting ε cells, and pancreatic polypeptide-secreting γ cells; (ii) the exocrine lineage, including acinar cells that secrete digestive enzymes into the small intestine; and (iii) the ductal lineage, which includes duct cells that secrete bicarbonate to balance pH in the digestive tract. The first wave of pancreas development is termed the 'primary transition', where there is rapid expansion of $Pdx1^{+}Sox9^{+}$ progenitor cells. At this time, the developing pancreas epithelium becomes segregated into the $Cpa1^{+}Sox9^{-}$ multipotent tip progenitor cells and $Cpa1^{-}Sox9^{+}$ bipotent trunk progenitor cells that give rise to the endocrine and duct cell lineages [16,18]. Subsequently, the pancreas undergoes a secondary transition around E13.5–E15.5 in the mouse in which differentiation occurs and exocrine, ductal, and endocrine cell numbers are dramatically expanded [14]. During this transition, multipotent $Ptf1a^{+}Cpa1^{+}$ tip progenitors become restricted to the exocrine lineage and cells start to express the mature exocrine marker amylase, while expression of Cpa1 and Ptf1a persists only in these cells [18]. Similarly, Sox9 is initially expressed in all pancreas progenitors, but becomes first restricted to bipotent progenitors, and then, during the secondary transition, to the ductal cell fate [19]. All five islet endocrine cell types are also derived from these bipotent trunk progenitors that activate the transcription factor *Ngn3* [18]. Ngn3 in turn induces a cascade of transcription factors, including *Neurod1*, *Arx*, *Mafa*, *Mafb*, *Rfx6*, and others, which help specify endocrine progenitors into the different endocrine cell types and drive their maturation [14].

Glossary

Bromodomain: a protein domain that recognizes acetylated lysine residues of histones.
Chromatin structure: chromatin can be modified with histone variants, DNA methylation, and various post-translational modifications to create several different chromatin states (Box 1).
***Cis*-regulatory loci:** regions of the genome that regulate gene activity on the same DNA strand (in *cis*). These include promoters, enhancers, insulators, and silencers.
Corepressor of RE1 Silencing Transcription factor (CoREST) complex: involved in histone deacetylation via Hdac1 and Hdac2, and contains the corepressor CoREST protein.
CpG: refers to locations in the genome where Cytosine and Guanine (CpG) nucleotides are adjacent to each other. A higher proportion of unmethylated CpG regions are found in the genome near transcriptional start sites and these are known as CpG islands.
Dedifferentiate: a process whereby fully differentiated cell types lose their mature identity and reverse towards more progenitor cell types.
Imitation SWI (ISWI) complex: involved in ATP-dependent nucleosome remodeling and contains an ISWI ATPase.
Long noncoding RNAs (lncRNAs): a class of nonprotein-coding transcripts that frequently function as scaffold proteins and regulate gene expression through chromatin and protein complex interactions.
Nonobese diabetic (NOD) mouse model: a model of T1DM that develops spontaneous immune cell infiltration of the pancreatic islets called insulitis.
Nucleosome remodeling: can refer to the movement, eviction, or addition of nucleosomes along a DNA strand.
Nucleosome Remodeling histone Deacetylase (NuRD) complex: involved in both ATP-dependent nucleosome remodeling and histone deacetylation.
Polycomb Group protein complex (PcG): chromatin remodeling complexes involved in H3K27me3-mediated chromatin repression.
Polycomb and trithorax response elements (PREs/TREs): *cis*-regulatory sequences of the