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Transcriptional Regulation of the Pancreatic Islet: Implications for Islet Function

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Abstract Islets of Langerhans contain multiple hormone-producing endocrine cells controlling glucose homeostasis. Transcription establishes and maintains islet cellular fates and identities. Genetic and environmental disruption of islet transcription triggers cellular dysfunction and disease. Early transcriptional regulation studies of specific islet genes, including insulin (*INS*) and the transcription factor *PDX1*, identified the first *cis*-regulatory DNA sequences and *trans*-acting factors governing islet function. Here, we review how human islet "omics" studies are reshaping our understanding of transcriptional regulation in islet (dys)function and diabetes. First, we highlight the expansion of islet transcript number, form, and function and of DNA transcriptional regulatory elements controlling their production. Next, we cover islet transcriptional effects of genetic and environmental perturbation. Finally, we discuss how these studies' emerging insights should empower our diabetes research community to build mechanistic understanding of diabetes pathophysiology and to equip clinicians with tailored, precision medicine options to prevent and treat islet dysfunction and diabetes.

Keywords Genome-wide association study (GWAS) · Promoter · Broad H3K4me3 domain (BD) · Enhancer · Stretch/super enhancer (SE) · Chromatin interaction analysis by paired end tag sequencing (ChIA-PET) · Chromatin immunoprecipitation (ChIP)-seq · RNA-seq · Islet · Type 1/2 diabetes (T1D/T2D) · Chromatin · Expression quantitative trait locus (eQTL) · Splicing quantitative trait locus (sQTL) · Allele-specific expression (ASE) · Allele-specific expression quantitative trait locus (aseQTL) · Single nucleotide polymorphism (SNP) · Inflammation · Oxidative stress · Endoplasmic reticulum (ER) stress

Introduction

The islets of Langerhans are clusters of at least five cell types—alpha, beta, delta, epsilon, and pancreatic polypeptide (PP)—that, together, comprise ~1–2 % of the pancreas and execute pancreatic endocrine functions. The DNA in each of these cells is largely identical, yet they are wired to complete distinct and complementary functions to maintain tight glycemic control. Until recently, most of our understanding of islet composition, physiology, and pathophysiology has been driven by animal model studies (mostly mouse and rat). Recent comparative analyses have revealed species-specific differences in the cellular architecture/composition of islets [1, 2], gene expression programs [3], and insulin secretion properties [2, 3], emphasizing the importance of studying and understanding physiologic processes and pathophysiologic responses in human islets alongside model systems. Moreover, human genetic variants affecting islet (dys)function may not exist or be properly modeled in other species.

Islet endocrine cell type composition varies both between individuals and between pancreatic sub-regions of the same individual, but averages 54 % (range 28–75 %) insulin-producing beta cells, 35 % (range 10–65 %) glucagon-secreting alpha cells,

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11 % (1–22 %) somatostatin-secreting delta cells, and very few epsilon and PP cells [1, 2]. As might be expected, based on morphologic fluctuations, islet function also varies between individuals [4, 5]. Many of the rare genetic differences that cause monogenic islet disorders, such as congenital hyperinsulinemia (CHI), permanent/transient neonatal diabetes mellitus (PNDM/TNDM), and maturity onset diabetes in the young (MODY), are protein-coding changes in islet transcription factors (TFs) or non-coding changes that affect islet transcriptional regulation. Islet transcriptional dysregulation is also implicated by types 1 and 2 diabetes genetic susceptibility studies [5, 6••, 7, 8••].

Transcription is a fundamental cellular process that governs cell fate choices in developing cells and myriad physiologic and pathophysiologic responses in mature cells. RNA Polymerase 2 (Pol2) transcribes genes encoded by the cell's DNA into various messenger RNA (mRNA) molecules. Early transcriptional regulation studies demonstrated the importance of *trans*-acting factors (DNA binding proteins) binding to *cis*-acting DNA sequence motifs immediately prior to a gene's transcriptional start site in transcriptional control. Recent studies indicate that sequences/sites distant from promoters also mediate cell type-specific transcription. Moreover, they indicate that molecular features not specifically predicted by DNA sequence motifs, such as local DNA shape and DNA accessibility and long-range folding/packing in the nucleus, also influence transcriptional regulation. Together, these features control the recruitment and/or activation of Pol2 transcriptional complexes at specific genes. Genetic (e.g., sequence variation) or epigenetic (e.g., chromatin remodeling) perturbation of these features can disrupt normal transcription, contributing to cellular dysfunction and disease.

Here, we review how next generation sequencing-based molecular profiling technologies performed on human islets over the past 5 years is transforming our understanding of transcriptional (dys)regulation in human islet (dys)function and disease. Islet transcriptome analyses by RNA sequencing (RNA-seq) have uncovered greater diversity of transcripts, in form, number, and function, than previously estimated. Epigenomic analyses have discovered tens of thousands of new transcriptional regulatory regions that coordinately control islet transcriptional output in resting, stimulated, and stressed states. Studies of islet genomes, epigenomes, and transcriptomes from multiple people are providing insights into genetic differences that alter transcriptional regulation to contribute to islet dysfunction and disease. Studies comparing diabetic and non-diabetic islet transcriptomes have identified abnormal transcriptional features; these have emphasized the need to better understand the transcriptional consequences of specific islet stresses, such as inflammation, endoplasmic reticulum stress, and oxidative stress. These studies have facilitated new insights into the genetics and molecular mechanisms of islet (dys)function and disease and are fueling new opportunities for preventative and therapeutic approaches to diabetes treatment.

The Expanding Islet Transcriptome

How many genes are transcribed in the islets? Until recently, our understanding of the islet transcriptome was limited by technology of the time. Microarray-based expression analysis required existing knowledge about the location and structure of genes to design the nucleotide probe sequences to interrogate gene expression. In contrast, RNA sequencing (RNA-seq) does not require such a priori knowledge or design limitations and has enabled agnostic interrogation of the entire transcriptomes of cultured cells and tissues covering a wider range of expression level than microarrays [9]. RNA-seq of human islets and sorted constituent cells has dramatically expanded islet transcriptome catalog numbers, forms, and functions.

The first application of high throughput sequencing to catalog the pancreatic islet transcriptome identified approximately 21,000 transcripts, corresponding to 7600 genes [10]. Deeper sequencing of islets from multiple individuals followed to characterize the transcriptomes of intact islets [11–17•] and their dissociated, sorted, and purified constituent cells, notably insulin-producing beta cells and glucagon-producing alpha cells [11, 18]. These studies, performed on samples from different individuals and under different conditions, collectively suggest that as many as 50–60 % of known genes are modestly expressed in islets (10,883 genes with reads per kilobase per million mapped reads (RPKM) >1 and 17,175 genes with RPKM>0.5) [12•, 13••, 16]. These include several genes near DNA sequence variants (single nucleotide polymorphisms; SNPs) implicated by genome-wide association studies (GWAS) in genetic susceptibility to type 1 (T1D) and type 2 diabetes (T2D). These data support current views of the importance of islet (dys)regulation in T2D pathophysiology [19] and have rekindled interest in potential roles for islet transcriptional dysregulation in early or progressive pathophysiologic events leading to T1D [7, 20]. MicroRNA (miRNA) have also been profiled in human islets [21, 22]; a recent review describes their roles in post-transcriptional regulation and islet biology [23].

Initial RNA-seq analyses also hinted that several genes in islets and beta cells may undergo alternative splicing to form multiple, distinct transcripts (isoforms). Alternatively spliced transcripts contribute to cell type-specific gene functions and have been implicated in both physiologic and pathophysiologic events in cells. For example, alternative splicing of *TCF7L2* and *G6PC2* was implicated as a molecular consequence of their respective GWAS SNP risk alleles [24]. Analysis of human islet transcriptomes from 11 individuals suggests that 1000–2000 genes may undergo alternative splicing in islets [11]. Analysis of ~90 human islet transcriptomes linked alternative splicing control of 371 islet transcripts to a specific SNP, termed splicing quantitative trait loci (sQTL) [17•]. Thus, the islet transcriptome is quite diverse and this diversity

is, to at least some extent, under genetic control. Better understanding of these mechanisms in islets should provide insights into islet dysfunction and diabetes.

Long non-coding RNA transcripts (lncRNAs) are a newly identified class of transcripts with implications in many diseases. LncRNAs mediate diverse developmental and pathophysiologic processes ranging from imprinting and X inactivation to tumorigenesis (reviewed in [25]), but lncRNA cell type specificity and low sequence conservation between species has made it difficult to predict lncRNA functions and modes of action [26]. Fueled by epigenome and transcriptome profiling of multiple cell types and tissues over the past 5–6 years, the catalog of lncRNAs is continuously and rapidly expanding. They were first systematically identified [27] using three criteria: (a) histone 3 lysine 4 trimethyl (H3K4me3)-histone 3 lysine 36 trimethyl (H3K36me3) "active transcription" epigenetic marks that occur over regions of the genome devoid of gene annotations; (b) minimum transcript size of 200 nucleotides; and (c) minimal protein-coding potential. They are exquisitely cell type-specific; each RNA-seq study of a new cell type or tissue identifies new lncRNAs. Morán et al. [13••] applied the above lncRNA criteria to islet and FACS-sorted beta cell RNA-seq data to identify 1128 lncRNAs. Independent RNA-seq studies of human islets have identified a similar number of lncRNA (1297 [15]); however, only 25–30 % (n=349) of lncRNAs overlap between studies. It is unclear whether this discrepancy is due to technical differences in islet handling/data processing steps or to genetic differences between samples. Uniform and joint analysis of these datasets will help to rectify these apparent discrepancies and to identify the complete compendium of human lncRNAs in islet cells under baseline conditions.

Understanding molecular functions of newly identified islet lncRNAs is an important goal. The first islet lncRNAs were described in 2012, so it is still early to dissect their precise role(s) in islet cell development, identity, and (patho)physiology. Islet lncRNA expression patterns and genomic location suggest they may serve as important biomarkers or mediators of islet (dys)function and diabetes. Their expression is developmentally controlled, and a subset of them are glucose-responsive and associated with changes in HbA1c, a long-term measure of glucose control [13••, 17•]. Several are situated next to genes encoding islet TFs, including *PDX1*, *HNF1A*, *NEUROD1*, *MAFB*, *FOXA2*, *ISL1*, and *NKX* in the genome; others are co-expressed with islet TF and insulin secretion genes [17•]. LncRNA knockouts in beta cells or whole animals, coupled with additional profiling of lncRNA behavior under different stimulatory and stress conditions, should shed light on the functions of these new and exciting RNA species.

Identification of Islet Transcriptional Regulatory Elements

Initial islet transcriptional regulation studies focused on regions immediately upstream of transcription start sites. They also relied almost exclusively on in vitro reporter assays for insights into in vivo control. These studies made at least two important contributions to understanding islet transcriptional regulation. First, they identified DNA sequence motifs that regulate islet gene expression, including the A-box, C1, E1, and CRE sequences in the insulin gene promoter [28] and areas I–IV upstream of the *PDX1* gene [29, 30]. Second, they facilitated discovery of important islet TFs that bind these motifs, such as PDX1, MAFA, NeuroD, and FOXA2/HNF3Beta. However, detailed comparison of in vitro and in vivo Pdx1 binding to target promoters emphasized the importance of DNA accessibility and nuclear chromatin structure in dictating which sequences are actually TF bound and used in vivo by islets and beta cells [31].

In the nucleus of each cell, DNA is wrapped around histone octamers to form chromatin. Inactive (heterochromatin) and actively transcribed (euchromatin) chromatin regions of the genome exhibit different features, including varying degrees of openness/accessibility and distinct histone protein covalent modifications [32]. Euchromatin is more loosely packed and accessible to specific and general TFs. As such, active regulatory elements are accessible to enzymes like DNase I [33]. Histone modification patterns are used to stratify them into promoter, enhancer, and insulator elements [32, 34–38] (Fig. 1a).

Several techniques have been recently applied to functionally profile human pancreatic islets. DNase-seq and FAIRE-seq studies revealed approximately 100,000 [39] and 80,000 [40] open sites (referred to as "peaks") in islets, respectively. These two studies expanded the catalog of potential islet *cis*-regulatory elements from a few thousand promoters to tens of thousands of promoters and non-promoter (enhancer, insulator) elements. Together with genetic susceptibility studies, these data are uncovering the complexity of islet transcriptional regulation and emphasizing the importance of enhancer control of islet transcription programs in physiologic and pathophysiologic states. Table 1 summarizes tools contributed by multiple studies [12•, 39, 41•, 42•] to search, visualize, and retrieve the locations of epigenetic features, described below, in islets and other cell types.

Promoters

Promoters are *cis*-regulatory elements located adjacent to the transcriptional start sites (TSSs) of genes (Fig. 1a). They are necessary for transcription of a gene to occur, but recent genome-wide surveys of *cis*-regulatory elements suggest they may not be sufficient to direct cell type-specific or robust gene

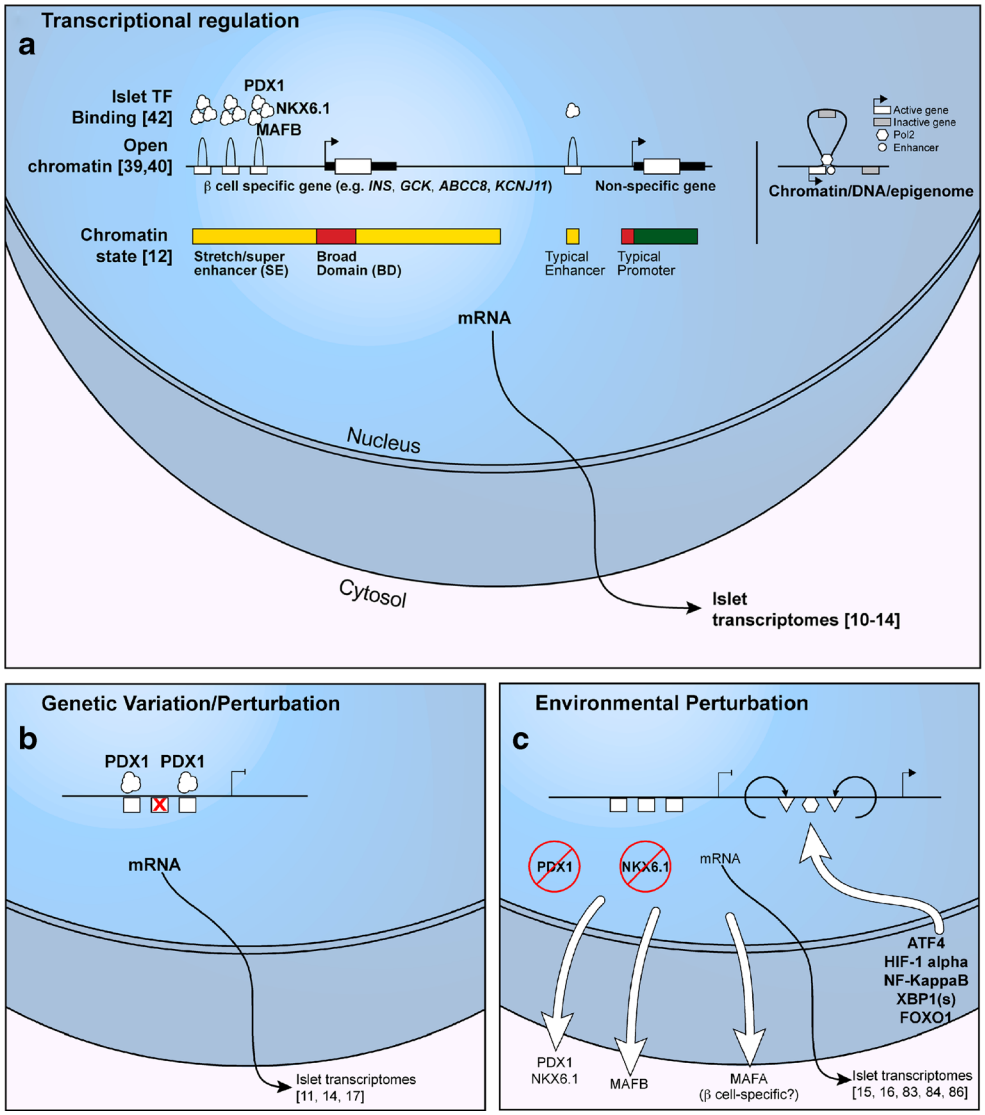

Fig. 1 Transcriptomic and epigenomic features of normal and perturbed islets. (**a**) Transcriptional regulatory features in islets. *Left* Open chromatin, islet transcription factor (TF) binding, and combinations of histone modification patterns (chromatin state) identify regulatory features in islets. Genes important for islet/beta cell identity and function (e.g., *INS*, *KCNJ11*, *ABCC8*, *GCK*) exhibit important epigenetic features, such as clustered sites of open chromatin (*humps*) and multiple islet TF binding (*cotton balls*), and extended enhancer (*yellow bars*) and promoter (*red bars*) chromatin states compared to features around a typical gene. *Green bar* indicates a "transcription elongation" state typically observed over non-specific, expressed genes. Right; 3D epigenomic analyses identify enhancer-target gene links, which can involve looping out/exclusion of the nearest gene (*gray rectangle*) on the linear genome to mediate 3D interactions between the enhancer (*white circle*) and target gene promoter (*white rectangle*). **b** Some genetic variants disrupt TF binding motifs (*red* "*X*"), abrogating protein binding (e.g., PDX1), reducing chromatin accessibility, and inactivating the gene. **c** Islets respond to perturbations such as oxidative stress, inflammation, and oxidative stress with nuclear translocation of several stress-responsive TFs (e.g., NF-KappaB, ATF4, XBP1(s), HIF1-alpha). These factors bind to new islet regulatory elements (*triangles*, *circle*) to activate the appropriate stress response genes (*arrowhead*). Islet TFs are inactivated and/or exported from the nucleus (*white arrows; red circles with slashes*), abandoning their binding sites (*rectangles*) and leading to gene inactivation

transcription. Targeted promoter studies to determine the mechanisms governing *INS* transcription were instrumental to identify several important factors regulating gene expression in beta cells and islets, including PDX1, NEUROD1, and MAFA and to define the *cis*-regulatory sequences they bind to exert their control [28].

Genome-wide, active promoters are enriched for histone 3 lysine trimethylation (H3K4me3). H3K4me3 chromatin immunoprecipitation-sequencing (ChIP-seq) identified approximately 14,000–18,000 promoter sites in the genome that are potentially active or poised for use in islets [12•, 18•, 39, 42•, 43]. In addition to cataloging islet promoters, these studies contributed the following insights about transcriptional regulation in islets. First, very few promoters are islet-unique. When compared with multiple other cell types, only ~1.5 % of promoters (n=256) were exclusively

Table 1 Islet regulatory element databases

Dataset	URL	Study
Islet open chromatin (DNAse-seq; "PanIslets" track)	http://www.genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=405493199_538GgMsR3I61PZAYyET9dQcNHIVk&c=chr6&g=wgEncodeOpenChromDnase	[39]
Broad H3K4me3 Domains (BDs)	http://bddb.stanford.edu	[41•]
Chromatin states from 10 cell types (including islets); Stretch Enhancers (SEs)	http://research.nhgri.nih.gov/manuscripts/Collins/islet_chromatin/	[12•]
Islet enhancer clusters (Islet Regulome)	http://www.isletregulome.org	[42•]

H3K4me3-positive in islets [39]. However, this set included several important genes for islet function such as the beta cell-specific hexokinase (*GCK*), the RNA binding protein, HuD, that regulates *INS* translation (*ELAVL4*) [44], and the zinc transporter, ZnT8, important for insulin granule assembly and secretion (*SLC30A8*). This observation is consistent with other studies [35, 45, 46] and suggests that cell type-specific (e.g., islet-specific) transcriptional control of gene expression is dictated by specific promoter use for only a small subset of genes. There is some discrepancy in the promoter architecture and putative transcriptional regulatory mechanisms at genes encoding major islet hormones such as insulin (*INS*), glucagon (*GCG*), and somatostatin (*SST*) [39, 42•, 43, 47]. Data from multiple groups suggest that these promoters are not highly enriched for the typical punctate H3K4me3 active promoter mark despite the genes being highly expressed [39, 43, 47]. Detailed *INS* locus analysis defined this region as an islet "open chromatin domain"—an 80-kb region encompassing the *INS*/*IGF2*/*TH* genes that does not exhibit punctate promoter marks, but rather widespread general openness, active histone modification patterns, and evidence of pervasive transcription throughout the locus [48].

Enhancers

Enhancers are DNA sequences in the genome that amplify or "enhance" transcription of a gene above baseline levels. They confer spatial and temporal specificity to promoter activity and gene expression in developing and mature cells and tissues. Enhancer features and specific examples, including the limb-specific enhancer controlling *SHH* expression and the beta-globin locus control region (LCR) controlling expression of multiple hemoglobin genes at different stages of development, have been elegantly reviewed recently [49]. Extensive epigenomic surveys of open chromatin by DNase-seq and histone modifications by ChIP-seq in hundreds of cell types have enabled the genome-wide and systematic identification of these elements and elucidated some of their general features [34–37, 45, 47, 50, 51]. Enhancer sites are typically exhibit histone 3 lysine 4 monomethylation (H3K4me1) enrichment. Active and poised enhancers are distinguished by presence or absence of H3 lysine 27 acetylation (H3K27ac), respectively [36, 37].

Islet ChIP-seq studies have identified 30,000–60,000 putative enhancers in islets [12•, 39, 42•, 43]. This is consistent with data from other cell types, such that enhancers outnumber promoters by two- to fourfold [34, 35, 47], and represents a dramatic expansion of the *cis*-regulatory landscape of islets. Islet enhancers are more cell-specific than islet promoters, suggesting they are key mediators of islet-specific transcriptional responses. Moreover, sequence variants contributing to variation in islet expression, function (e.g., fasting glucose), or risk of type 2 diabetes are significantly and specifically enriched to overlap islet enhancers [12•, 17•, 42•, 51].

Bigger is Better

Independent studies of genome-wide histone modification patterns in multiple cell types have revealed that both promoter and enhancer chromatin marks range in length from hundreds to tens of thousands of nucleotides [12•, 41•]. Broad domains (BDs; Fig. 1a) are the longest 5 % of contiguous H3K4me3 promoter marks (>4 kilobases; kb) [41•]. They mark promoters of genes of particular importance for cell-specific identity and function. For example, BDs mark promoters of genes encoding the pluripotency TFs (OCT4, SOX2, NANOG) specifically in embryonic stem cells. Approximately 1000–3000 genes are BD marked in a given cell type. This long epigenetic mark seems to reflect or govern stable, consistent gene expression rather than solely high levels of expression [41•]. Islets contain approximately 3500 BD-marked genes. BD-marked genes in islets include major TFs (e.g., *PDX1*, *MAFA*/*B*, *NKX6-1*/*2-2*), genes encoding key enzymes for glucose processing (*GCK*, *G6PC2*), and those regulating insulin production and secretion (*ELAVL4*, *KCNJ11*, *SLC30A8*, *PCSK1*/*2*, *CACNA1C*/*D*). The BD mark has also been used as a screening tool to identify novel genes that play important roles in cell identity and function [41•]. We expect that systematic screens of BD-marked genes in islets will uncover new gene(s) and pathway(s) controlling islet function.

Similar to broad domains, independent studies have identified a subset of enhancers in a cell that seem to govern the transcription of genes particularly important to cell identity and function. *Stretch enhancers* are the longest 5–10 % of

enhancer states (>3 kb long) in each cell type and are located near to or overlapping genes important for cell type-specific functions (Fig. 1a, left). *Super enhancers* (SEs) were originally defined as single or clustered sites in the genome bound by a disproportionate amount of cell type-specific master TFs and/or coactivator proteins [52, 53]. Subsequently, surveys of multiple cells identified super enhancers based on long stretches of H3K27ac "active enhancer" modifications [54•]. Although stretch and super enhancers (SEs) are not equivalent regulatory entities, they overlap at several loci and share important functional features: (i) they are highly cell type-specific and overlap locus control regions (LCRs)—complex regulatory regions dictating the developmental regulation of certain genes; (ii) they are associated with cell type-specific expression of genes important for cell type-specific functions; and (iii) they are enriched for SNPs associated with phenotypes and diseases affecting the relevant cell type (e.g., T2D or fasting glucose SNPs enriched in islet SEs) [12•, 54•]. In islets, they are comprised of clustered constituent open chromatin sites [39, 40] bound by multiple master islet TFs, such as PDX1, NKX6-1, FOXA2, and MAFB [42•]. These complex regulatory sites may function as regulatory hubs or transcription factories to coordinate transcriptional activity.

Connecting the Pieces

With transcriptional regulatory element "parts lists" in hand, a critical step is to assemble the components—enhancers, promoters, and insulators—into a detailed wiring diagram of the circuits that control transcriptional responses to stimulus and stress in the nucleus of islet cells (Fig. 1a, right). Connectivity maps provide mechanistic insights into cell type-specific transcriptional regulation [55–58] and link SNP-containing enhancers to their target genes [55, 59]. Felsenfeld and colleagues used 4C, a variation of the chromosome conformation capture technique (reviewed in [60]) to identify genes physically interacting or in close three-dimensional (3D) proximity with the *INS* promoter in islets. They discovered that the *SYT8* and *ANO1* genes, located approximately 300 kb and 68 megabases (Mb), respectively, from the *INS* promoter on the linear DNA, are close together in the 3D nucleus [61, 62]. Both interactions are islet-specific and functionally link *INS* transcription with that of two genes encoding a membrane protein (*SYT8*) and an ion channel (*ANO1*) important for insulin exocytosis. Glucose stimulation strengthened both interactions and enhanced *SYT8* and *ANO1* expression in islets. This suggests that these genes form glucose-responsive transcriptional co-regulatory units, supporting the transcription factory model introduced over 10 years ago [63].

The two- to fourfold excess of enhancers relative to promoters strongly suggests that this circuitry is more complex than a collection of single enhancer-promoter interactions. Clustered open chromatin sites [39, 40] and islet TF binding [42•] in SEs suggest that these elements form complex regulatory hubs or co-regulatory units, with multiple enhancers coordinately regulating one or more promoters. Ferrer and colleagues used 4C to identify several putative enhancers interacting with specific promoters, including *ISL1*, *PDX1*, and *MAFB* [42•]. They confirmed interaction between known regulatory elements and their target promoter (e.g., regions I–IV upstream of *PDX1* [29, 30]) and discovered novel interactions, some of which extended over 1 Mb from the promoter. Because these are bulk islet interactions, we do not know if they are present in all islet cell types or if all or a subset of these interactions is formed in a specific islet cell type (e.g., beta cells). Overall, however, these data support a model wherein multiple enhancers contact a gene promoter to coordinately regulate its activity in islets. These examples illustrate the utility and importance of 3D epigenome approaches to better understand transcriptional regulation in islets and to assign enhancers to their target genes. It will be important (1) to expand upon these targeted analyses to identify comprehensively all promoter-promoter, promoter-enhancer, and enhancer-enhancer interactions at high-resolution using techniques such as ChIA-PET [64] or Capture-C [65]; and (2) to assign these interactions to their specific endocrine cell type to better understand cell type-specific (e.g., alpha, beta, delta cell) connectivity, especially those of BDs and SEs.

Perturbed Transcriptional Regulation in Islet Dysfunction and Diabetes

Effects of Individual Genetic Variation on Islet Transcription

DNA sequence variants that alter islet transcriptional programs lead to both rare and common forms of diabetes. Over half of the genes containing disease-causing mutations in patients with PNDM, TNDM, MODY, and CHI encode islet TFs. These include well-known genes such as *PDX1*, *HNF1A*, *HNF1B*, *HNF4A*, and *NEUROD1*. Recent exome sequencing of neonatal diabetes patients has identified mutations in two additional TF genes, *GATA4* [66] and *GATA6* [67]. Exome sequencing of PNDM, TNDM, MODY, and CHI patients with undiagnosed mutations is ongoing and is almost certain to identify mutations in additional, perhaps unexpected, TFs. Understanding the *cis*-regulatory elements bound by these factors and their target genes is necessary to better understand the pathophysiologic consequences of their disruption. Such an endeavor may define new sites that could contain disease-predisposing mutations.

Although less numerous than protein-coding mutations, rare variants altering islet *cis*-regulatory elements have been identified in families with monogenic islet disorders (Table 2). Promoter mutations in *KCNJ11* and *ABCC8* were identified in

Table 2 Genetic variants altering islet transcriptional regulatory elements

Target gene (GWAS locus)	SNP	Islet regulatory element	Alleles	Risk/effect allele	eQTL/aseQTL?	Transcriptional effect	Molecular genetic effect	Physiologic effect/ association	Reference
Rare variants									
GCK	−71 G/C	Promoter	G/C	C	ND	Down	Loss of SP1 binding	Fasting hyperglycemia	[72]
ABCC8	−64 C/G	Promoter	C/G	G	ND	ND	ND	CHI	[68]
HFN4A	−146 T>C	Promoter (P2)	T/C	C	ND	ND	Mutated PDX1 binding site	MODY	[70]
HFN4A	−192 C>G	Promoter (P2)	C/G	G	ND	ND	ND	Impaired GSIS; MODY-X; GDM; T2D	[69]
HFN4A	−136 A>G	Promoter (P2)	A/G	G	ND	ND	ND	MODY-like	[71]
HFN4A	−169 C>T	Promoter (P2)	C/T	T	ND	ND	ND	MODY-like	[71]
BLK	chr8:11,459,364; chr8: 11,459,531 (NCBI Build 36.1)	Putative Enhancer/ Insulator	T/G;G/T	G;T	ND	ND	Decreased reporter enhancer activity	MODY	[73]
KCNJ11	88 G-T	Promoter	G/T	T	ND	ND	ND	CHI	[68]
Common variants									
TCF7L2	rs7903146	Enhancer	C/T	T	eQTL	Up	Increased open chromatin; increased reporter enhancer activity	T2D	[14, 39, 40, 77]
MTNR1B	rs10830963	Enhancer	C/G	G	eQTL	Up	ND	T2D	[17•, 81]
CAMK1D	rs12779790; rs11257655	Enhancer	A/G; C/T	G;T	eQTL	Up	Increased FOXA1, FOXA2 binding	T2D	[14, 80]
ARAP1	rs11603334	Promoter	C/T	T	eQTL	Up	Decreased PAX6, PAX4 binding	Decreased proinsulin; T2D	[78]
G6PC2	rs13431652	Promoter	G/A	A	eQTL	Up	Increased NF-Y binding	Increased fasting plasma glucose	[24]
G6PC2	rs2232316	Promoter	A/G	A	eQTL	Up	Increased FOXA2 binding	Increased fasting plasma glucose	[24]
G6PC2	rs573225	Promoter	A/G	A	eQTL	Down	Increased FOXA2 binding	Increased fasting plasma glucose	[24]
KCNJ11	rs5912	Promoter	C/T	T	aseQTL; eQTL	Down	ND	T2D	[11, 14]
CLEC16A	rs12708716	Enhancer (?)	A/G	G	eQTL (Islets)	Down	ND	T1D; decreased beta cell function (HOMA-B); increased HbA1c	[20]
CTSH	rs3825932	Promoter	T/C	T	eQTL	Down	ND	T1D; IGT	[76]
ZFAND3	rs58692659	Enhancer	A/G	A	ND	ND	Disrupted NEUROD1 binding	T2D	[42•]
ADRA2A	rs553668	3 UTR	A/G	A	eQTL	Up	ND	Decrease insulin secretion; Decrease GSIS; T2D	[4, 78, 82••]

ND not determined, *e/aseQTL* expression/allele-specific expression quantitative trait locus, *GSIS* glucose-stimulated insulin secretion, *CHI* congenital hyperinsulinemia, *MODY* maturity onset diabetes of the young, *T2D* type 2 diabetes, *T1D* type 1 diabetes, *IGT* impaired glucose tolerance

CHI patients [68]. Reporter assays indicate that these mutations each decreased promoter activity by ~60 %. Similarly, inactivating promoter mutations in *HNF4A* P2 were identified for both MODY1, MODY-like, and gestational diabetes patients [69–71], and in the beta cell *GCK* promoter in patients with fasting hyperglycemia [72]. Intergenic mutations in the *BLK* locus, which decreased reporter gene activity, were identified as causative for MODY9 [73]. Inspection of islet chromatin maps [12•, 39] indicate that the location of at least one of the described MODY9 mutations overlaps a putative islet enhancer also bound by CTCF, suggesting this rare variant could disrupt its function. Finally, six different recessive mutations in a developmental enhancer 25-kb downstream of the *PTF1A* gene (pancreas-specific transcription factor 1a) were identified in 10 families with pancreatic agenesis [74•]. We expect that the recent documentation of hundreds of thousands of enhancer elements in pancreatic islets and anticipated identification of hundreds of thousands of enhancer elements in various islet developmental precursor cell types will lead to the discovery of new, rare enhancer mutations contributing to monogenic diabetes and islet dysfunction disorders.

Genome-wide association study (GWAS) results suggest that common variant effects on islet transcription are important for islet (dys)function, T1D, and T2D. Massive consortia efforts have identified >100 regions of the human genome (loci) containing DNA sequence variants (SNPs) associated with genetic variation in glycemic traits related to islet (dys)function and susceptibility to both T1D and T2D. Approximately 90 % of these SNPs reside in noncoding regions of the genome, fueling the hypothesis that they disrupt transcriptional regulatory elements. T2D and glycemic trait GWAS SNPs are enriched in islet enhancers and several putative target genes are islet-expressed [12•, 14–17•, 42•]. T1D GWAS SNPs are enriched in lymphoid enhancers [75]. However, detailed functional analysis of T1D susceptibility genes *CLEC16A* [20] and *CTSH* [76] and the observation that multiple T1D-associated genes are also expressed in islets [11], [16] warrant continued attention to potential roles for aberrant islet transcriptional control in T1D pathophysiology.

The non-coding location of GWAS SNPs present at least four challenges to understanding their effect(s): (1) identifying the variant(s) responsible for the association; (2) understanding the molecular effect(s) of these variants; (3) identifying the gene(s) affected by these perturbations; and (4) determining the direction of the effect, i.e., gain- or loss-of-function, on the target gene. Several studies have begun to address these challenges. Table 2 summarizes our collective knowledge and reflects the per-locus variability in our understanding of common variant effects on islet transcription. Both molecular genetic and islet transcriptional consequences of SNP risk alleles have been deciphered for a handful of T2D GWAS loci, including *TCF7L2* [77], *SLC30A8*, *ADRA2A* [4, 78], *G6PC2* [24], *ARAP1* [79], and *CAMK1D*/*CDC123* [14, 80]. For loci such as *MTNR1B* [81], *CLEC16A* [20], *CTSH* [76], and *ZFAND3* [42•], either the transcriptional or the molecular genetic consequence of the SNP allele has been determined.

Recently, Groop and colleagues conducted microarray and RNA-seq studies of islets from 63 and 89 organ donors, respectively [14, 17•]. They found that expression of 640 genes is modulated by SNPs, including a subset associated with T2D and glycemic traits (Table 2). Additionally, they discovered ~1100 additional genes exhibiting allele-specific expression. Together, expression of approximately 1700 islet genes appears to be modified by genetic variation; this aligns with the median number of genes (1742) harboring at least one allele-specific expression SNP in an independent study [11]. Moreover, putative GWAS SNP target genes exhibited evidence of allelic islet expression (15/23 T1D, 20/28 T2D, and 15/18 glycemic trait genes) [11]. Further efforts to elucidate the molecular mechanisms controlling these islet expression and allelic expression differences should provide context to the pathophysiologic events and guide therapeutic strategies.

The motivation for these targeted and genome-wide analyses is to build more precise predictive risk models and prevention strategies, to identify diagnostic molecular markers, and to develop new and more precise therapeutic approaches to prevent and treat islet dysfunction and diabetes. Genome-informed modalities have been developed for a subset of MODY and neonatal diabetes patients and have impacted their prognosis and treatment [8••]. MODY patients with *HNF1A* and *HNF4A* mutations respond particularly well to low-dose sulfonylurea therapy, whereas those with *GCK* mutations are best left untreated. Neonatal diabetes patients with activating *KCNJ11*/*ABCC8* mutations can be effectively treated with high-dose sulfonylureas. Studies of rs553668, a GWAS SNP in *ADRA2A* associated with impaired beta cell function and T2D, provide an exciting example of translating common genetic variant association into molecular mechanism of action, physiologic consequences, and genotype-based treatment [4], [78, 82••]. *ADRA2A* encodes an adrenergic receptor that mediates adrenergic suppression of glucose-stimulated insulin secretion (GSIS) in islets [78]. The rs553668 risk allele leads to *ADRA2A* overexpression in islets and impaired insulin secretion in risk allele carriers [4, 78]. Administration of the adrenergic receptor antagonist yohimbine did not affect insulin secretion in non-risk individuals, but it improved insulin secretion in risk allele carriers to levels seen in the non-risk individuals [82••].

Environmental Effects on Islet Transcription

Studies comparing T2D and non-diabetic islet and beta cell transcriptomes have detected differences in hundreds of mRNA and several miRNA [14, 83–85]. Few differentially expressed genes are overlapping between studies. This likely reflects a combination of biological differences (organ donor

characteristics (e.g., race, genotype, sex, weight, age, and cause of death), duration of diabetes and degree of blood sugar control, and biological sample attainment and processing (enzymatic isolation of islets vs. LCM). However, on a pathway level, the studies consistently identified aberrant transcription of components of (1) core islet/beta cell function pathways, such as glucose sensing, insulin receptor signaling, glycolysis/beta oxidation, and glucose stimulated insulin secretion; (2) stress response pathways such as oxidative stress; and (3) islet TFs. These studies implicate transcriptional dysregulation of key pathways as a feature of islet dysfunction and T2D; more studies will be necessary to determine the causative nature of these changes.

Several studies have sought to understand the transcriptional consequences of early pathophysiologic events leading to islet dysfunction, T1D, and T2D. Stress response pathways postulated as early mediators of beta cell failure and death include inflammation, hypoxia/oxidative stress, and endoplasmic reticulum (ER) stress [8••]. All of these responses lead to changes in localization and/or activity of TFs in the islet (Fig. 1c). Inflammation and ER stress mediate transcriptional changes in islets via induction and nuclear localization of TFs like NF-kappaB, CHOP, XBP1s, ATF4, and ATF6. Hypoxia leads to HIF1alpha-/HIF1beta-mediated transcription. Oxidative stress causes export or inactivation of islet TFs such as PDX1, NKX6.1, and MAFA; these factors are also compromised in T2D islets [86•]. Few studies have assessed the comprehensive transcriptome changes using RNA-seq. 1325 genes were differentially expressed and 3525 were alternatively spliced after acute (48 h) exposure of human islets to the free fatty acid palmitate (modeling lipotoxicity), including 11/59 T2D GWAS candidate genes expressed in islets [15]. Similarly, exposure of islets to proinflammatory cytokines altered the expression and splicing of 3065 and 6875 genes, respectively [16]. Although these acute experimental exposures may not accurately reflect the precise changes in vivo, they provide a basis for understanding transcriptional consequences of islet damage and may identify pathways mediating pathophysiologic processes in T1D and T2D.

Conclusions

Islet "omics" studies have uncovered extensive diversity and complexity of the transcripts produced and also of the *cis*-regulatory elements controlling their production. Recent studies, highlighted in this review, have built a compendium of transcripts and regulatory elements and are working to assemble individual components into islet *cis*-regulatory transcriptional regulatory networks. They are building insights into the individual impact of either genetic variation or environmental perturbation of transcriptional control on islet physiology and pathophysiology. Diverse transcriptional features (regulatory element use, transcript levels, splicing) in islets are linked to genotype. Continued progress to identify the target gene(s) and direction of effect (gain-of-function or loss-of-function) of GWAS and other key regulatory SNPs is inevitable. Thus, we expect that additional stories akin to *ADRA2A* will emerge in the coming years. Studies integrating both genetic and environmental contributions to islet dysfunction are needed to realize precision medicine (prevention, monitoring, and treatment) approaches to islet dysfunction and diabetes. Finally, we anticipate that epigenomic and transcriptomic analysis of single cells or stratified islet subpopulations will provide more precise understanding of the cell type-specific (e.g., alpha, beta, delta) effects of genetic and environmental perturbation, which should impact pathophysiologic understanding and therapeutic approaches for islet dysfunction and diabetes.

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Compliance with Ethics Guidelines

Conflict of Interest Michael L. Stitzel, Ina Kycia, Romy Kursawe, and Duygu Ucar declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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