JNK signaling, thereby allowing neurons to tolerate potentially neurotoxic JNK signaling (Fig. 4I). Furthermore, Fbw7 has tumor suppressor function and is mutated in a high percentage of endometrial cancers with high cyclin E expression (*23*). Accumulation of the phosphorylated, transcriptionally active form of c-Jun in tumors that lack Fbw7 may contribute to cancer development.

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Control of Pancreas and Liver Gene Expression by HNF Transcription Factors

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The transcriptional regulatory networks that specify and maintain human tissue diversity are largely uncharted. To gain insight into this circuitry, we used chromatin immunoprecipitation combined with promoter microarrays to identify systematically the genes occupied by the transcriptional regulators HNF1α, HNF4α, and HNF6, together with RNA polymerase II, in human liver and pancreatic islets. We identified tissue-specific regulatory circuits formed by HNF1α, HNF4α, and HNF6 with other transcription factors, revealing how these factors function as master regulators of hepatocyte and islet transcription. Our results suggest how misregulation of HNF4α can contribute to type 2 diabetes.

Gene expression is controlled by transcriptional regulatory proteins, which bind specific DNA sequences and recruit cofactors and the transcription apparatus to promoters (*1–3*). Genome-wide analysis methods have been used recently to determine how most transcriptional regulators encoded in *Saccharomyces cerevisiae* are associated with the genome in living yeast cells and to model the transcriptional regulatory circuitry of these cells (*4*). These methods have also been used in human tissue-culture cells to identify target genes for several transcriptional regulators (*5–7*). Genome-scale analysis methods have yet to be used to determine how transcriptional regulators control the global gene expression programs that characterize specific tissues.

The liver and pancreas have long been the subject of studies to understand how organs develop and are regulated at the transcriptional level (*8–12*). The transcriptional regulators HNF1α (a homeodomain protein), HNF4α (a nuclear receptor), and HNF6 (a member of the onecut family) operate cooperatively in a connected network in the liver, but less is known about the structure of this regulatory network in human pancreatic islets. All three transcriptional regulators are required for normal function of liver and pancreatic islets (*13–18*). Mutations in HNF1α and HNF4α are the causes of the type 3 and type 1 forms of maturity-onset diabetes of the young (MODY3 and MODY1), a genetic disorder of the insulin-secreting pancreatic beta cells characterized by the onset of diabetes mellitus before 25 years of age and an autosomal dominant pattern of inheritance (*19*). Genome-scale analysis to find the pancreatic islet genes whose expression is regulated by these transcription factors in normal beta cells could provide insights into the molecular basis of the abnormal beta cell function that characterizes MODY. We identified the genes occupied by the transcription factors HNF1α, HNF4α, and HNF6 in hepatocytes and pancreatic islets, and we identified the genes transcribed in each tissue by determining the genomic occupancy of RNA polymerase II. We used this information to begin to map the transcriptional regulatory circuitry in these tissues.

We first used genome-scale location analysis (*20*) to identify the promoters bound by HNF1α in human hepatocytes and pancreatic islets isolated from tissue donors (Fig. 1A). For each tissue, HNF1α-DNA complexes were enriched by chromatin immunoprecipitation (ChIP) in three separate experiments. We constructed a custom DNA microarray containing portions of promoter regions of 13,000 human genes (Hu13K array). We targeted the region spanning 700 base pairs upstream and 200 base pairs downstream of transcription start sites for the genes whose start sites are best characterized on the basis of the National Center for Biotechnology Information (NCBI) annotation (*20*). Although many enhancers are present at more distant locations, most known transcription factor binding-site sequences occur within these start-site proximal regions.

The results of these genome location experiments revealed that HNF1α is bound to at least 222 target genes in hepatocytes, representing 1.6% of the genes on the Hu13K array (Table 1) (*20*). This result was verified with independent, conventional ChIP experi-

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ments, which suggest that the frequency of false positives in genome-scale location data with gene-specific regulators is no more than 16% when our threshold criteria were used (*20*). The genes that we found to be occupied by HNF1α in primary human hepatocytes encode products whose functions represent a substantial cross section of hepatocyte biochemistry. The results confirm that HNF1α contributes to the transcriptional regulation of many of the central rate-limiting steps in gluconeogenesis and associated pathways. HNF1α also binds to genes whose products are central to normal hepatic function, including carbohydrate synthesis and storage, lipid metabolism (synthesis of cholesterol and apolipoproteins), detoxification (synthesis of cytochrome P450 monooxygenases), and synthesis of serum proteins (albumin, complements, and coagulation factors).

We next identified HNF1α target genes in human pancreatic islets (Table 1) (*20*). HNF1α occupied the promoter regions of 106 genes (0.8% of the Hu13K array promoters) in islets, 30% of which were also bound by HNF1α in hepatocytes (Fig. 1B). In islets, fewer chaperones and enzymes are bound by HNF1α than in hepatocytes, and the receptors and signal transduction machinery regulated by HNF1α vary between the two tissues.

HNF1α has previously been implicated in the regulation of many genes in hepatocytes and islets (*13*, *16*, *20*) (table S4). The direct genome binding data reported here confirmed many but not all of these genes. The difference may be due, at least in part, to our stringent criteria for binding in the genome-scale data, which enhances our confidence in the direct target genes identified by location analysis, but likely underestimates the actual number of targets in vivo. Furthermore, although the proximal promoter regions printed on the array contain a number of transcription factor binding sequences, many genes are also regulated by more distal promoter elements and enhancers that are not present on the Hu13K array.

We also identified the promoters bound by HNF6 in human hepatocytes and pancreatic islets using genome-scale location analysis (Fig. 1B and tables S5 and S6) (*20*). HNF6 was bound to at least 227 genes in hepatocytes and 189 genes in pancreatic islets, representing 1.7 and 1.4% of the promoters on the array, respectively. About half of the promoters occupied by HNF6 were common to the two tissues, including a number of important cell cycle regulators such as cyclin-dependent kinase 2 (*20*).

Genome-scale location analysis revealed surprising results for HNF4α in hepatocytes and pancreatic islets (Fig. 1B). The number of genes enriched in HNF4α ChIPs was much larger than observed with typical site-specific regulators. HNF4α was bound to about 12% of the genes represented on the Hu13K DNA microarray in hepatocytes and 11% in pancreatic islets. No other transcription factor that we have profiled in human cells has been observed to bind more than 2.5% of the promoter regions represented on the Hu13K array.

Six independent lines of evidence indicate that the HNF4α results are not due to poor antibody specificity or errors in the microarray analysis; this evidence supports the view that HNF4α is associated with an unusually large number of promoters in hepatocytes and pancreatic islets (*20*). First, essentially identical results were obtained with two different antibodies that recognize different portions of HNF4α. Second, Western blots showed that the HNF4α antibodies are highly specific. Third, we verified binding at more than 50 randomly selected targets of HNF4α in hepatocytes by conventional gene-specific ChIP. Fourth, when antibodies against HNF4α were used for ChIP in control experiments with Jurkat, U937, and BJT cells (which do not express HNF4α), no more than 17 promoters were identified in each cell line by our criteria, which is well within the noise inherent in this system. Fifth, when preimmune antibodies from rabbit and goat (the two different antibodies to HNF4α came from rabbit and goat) were used in control experiments in hepatocytes, the number of targets identified was within the noise. Finally, if the HNF4α results are correct, then we would expect that the set of promoters bound by HNF4α should be largely a subset of those bound by RNA polymerase II in each tissue; we found that this is the case. We conclude that HNF4α is a widely acting transcription factor in these tissues, consistent with the observation that it is an unusually abundant and constitutively active transcription factor (*11*).

We next identified the genes represented on the Hu13K microarray that are actively transcribed in hepatocytes and pancreatic islets to determine the fraction of actively transcribed genes that are bound by HNF4α (Fig. 1C). It is difficult to determine the transcriptome of these tissues accurately by profiling transcript levels with DNA microarrays. Transcript profiling requires a reference RNA population against which a tissue RNA population can be compared, and there are limitations to generating appropriate reference RNA. To circumvent this limitation, we exploited the fact that RNA polymerase II occupies the set of protein-coding genes that are actively transcribed in eukaryotic cells. Location analysis with RNA polymerase II antibodies can identify these actively transcribed genes (*7*, *21*). We found that 23% of the genes on the Hu13K array (2984 genes) were bound by RNA polymerase II in hepatocytes, and 19% (2426 genes) were bound by RNA polymerase II in islets (*20*). The sets of genes occupied by RNA polymerase II in hepatocytes and islets overlapped substantially (81% overlap, relative to islets), consistent with the relatedness of the two tissues (*22*). As expected, the majority of genes occupied by HNF4α in hepatocytes and pancreatic islets (80 and 73%, respectively) were also occupied by RNA polymerase II. Notably, of the genes occupied by RNA polymerase II, 42% (1262 out of 2984) were bound by HNF4α in hepatocytes and 43% (1047 out of 2426) were bound by HNF4α in islets (Fig. 1C). By comparison, only 6 and 2% of RNA polymerase II–enriched promoters were also bound by HNF1α in hepatocytes and islets,

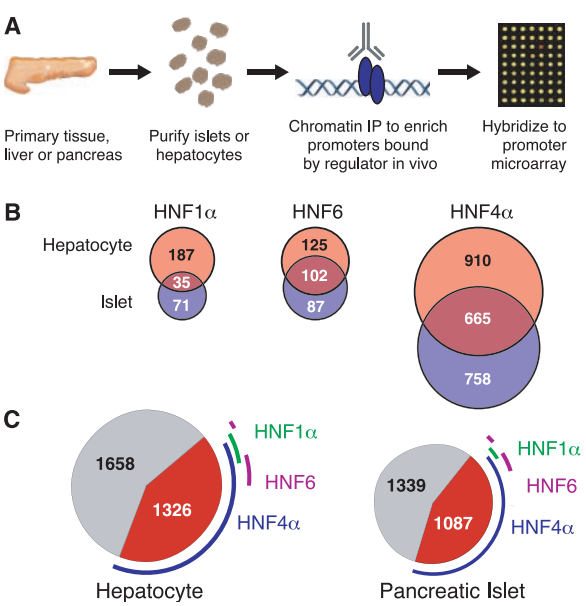

Fig. 1. Genome-scale location analysis of HNF regulators in human tissues. (**A**) Hepatocytes and pancreatic islets were obtained from tissue distribution programs. These cells were treated with formaldehyde to covalently link transcription factors to DNA sites of interaction. Cells were harvested, and chromatin in cell lysates was sheared by sonication. The regulator-DNA complexes were enriched by ChIP with specific antibodies, the cross-links were reversed, and enriched DNA fragments and control genomic DNA fragments were amplified with ligation-mediated polymerase chain reaction. The amplified DNA preparations, labeled with distinct fluorophores, were mixed and hybridized onto a promoter array. (**B**) Venn diagram showing the overlap of HNF1α-, HNF6-, and HNF4α-bound promoters in hepatocytes (top, red circles) and pancreatic islets (bottom, blue circles). (**C**) The collection of genes occupied by RNA polymerase II in hepatocytes is displayed as a circle, with the genes bound by HNF1α, HNF6, and/or HNF4α outlined collectively in red as a fraction of the chart. The relative contributions of HNF1α (green), HNF6 (purple), and HNF4α (blue) are shown as framing arcs.

respectively. The occupancy by HNF4α of a substantial fraction of expressed genes suggests that abnormal levels of HNF4α could impair glucose-stimulated insulin secretion in pancreatic beta cells by affecting a large portion of the transcriptome rather than through misregulation of a specific downstream target gene.

Previous studies indicate that HNF1α, HNF4α, and HNF6 are at the center of a network of transcription factors that cooperatively regulate numerous developmental and metabolic functions in hepatocytes and islets (*9*, *15*, *17*). Our systematic analysis of the direct in vivo targets of these factors considerably expands our understanding of the regulatory network in primary human tissues (Fig. 2A). A comparison of the regulatory network in these two tissues reveals that HNF1α, HNF4α, and HNF6 occupy the promoters of genes encoding a large population of transcription factors and cofactors (*20*). The precise set of transcription factor genes oc-

Table 1. Partial list of proximal promoters occupied by HNF1α in human hepatocytes and pancreatic islets. These genes were assigned to functional categories using the program ProtoGo (www.protogo.cs.huji.ac.il). Genes not in this automated Gene Ontology database were assigned on the basis of NCBI information. Four genes are shown for each tissue/category combination; for some combinations, fewer than four promoters qualified as targets. Hypothetical and functionally uncharacterized genes are not shown. A complete list of bound genes, including their accession numbers, is available (*20*) (tables S2 and S3). HGF, hepatocyte growth factor; Ubiq.-cyt., ubiquinol-cytochrome; IGF insulin-like growth factor; GTP, guanosine 5′-triphosphate; CREB, cyclic adenosine monophosphate response element–binding protein; RAR, retinoic acid receptor; OAT, organic anion transporter; SNAP, N-ethylmaleimide–sensitive factor attachment protein.

Name	Description	Hepatocytes	Islets
Chaperone			
C4BPA	Complement 4 binding protein α	x	x
APCS	Amyloid P component	x	
F11	Coagulation factor XI	x	
C1S	Complement component 1s	x	
VTN	Somatomedin B	x	
Enzyme—hydrolase			
PGCP	Glutamate carboxypeptidase		x
GLA	Galactosidase, α		x
LIPA	Lipase A		x
SPO11	SPO11-like		x
PAFAH2	Platelet-activating factor 2	x	x
AADAC	Arylacetamide deacetylase	x	x
PS-PLA1	Phospholipase A1α	x	x
VNN3	Vanin 3	x	x
CPB2	Carboxypeptidase B2	x	
ANPEP	Alanyl aminopeptidase	x	
HGFAC	HGF activator	x	
ENPEP	Glutamyl aminopeptidase	x	
Enzyme—ligase			
MCCC1	Methylcrotonoyl-CoA carboxylase		x
GARS	Glycyl-tRNA synthetase	x	
TARS	Threonyl-tRNA synthetase	x	
Enzyme—lyase			
UROD	Uroporphyrinogen decarboxylase		x
PCK1	Phosphoenolpyruvate carboxykinase 1	x	
HPCL2	2-hydroxyphytanoyl-CoA lyase	x	
HAL	Histidine ammonia-lyase	x	
FH	Fumarate hydratase	x	
Enzyme—oxidoreductase			
COQ7	COQ7 coenzyme Q, 7		x
ADH4	Alcohol dehydrogenase 4		x
UQCRC2	Ubiq.-cyt. c reductase core protein II	x	x
CYB5-M	Cytochrome b5	x	x
CYP2E	Cytochrome P450, IIE	x	
CYB5	Cytochrome b-5	x	
HSD17B2	Hydroxysteroid dehydrogenase 2	x	
ADH1A	Alcohol dehydrogenase 1A	x	
Enzyme—transferase			
GCNT3	Glucosaminyl transferase 3		x
FNTB	Farnesyltransferase β	x	x
HNMT	Histamine N-methyltransferase	x	
GOT1	Aspartate aminotransferase 1	x	
UGT2B15	UDP glycosyltransferase 2B15	x	
GBE1	Glycogen branching enzyme	x	
Enzyme regulator			
SERPING1	C1-inhibitor	x	
SERPINA1	α-1-antitrypsin	x	
ITIH4	Inter-α inhibitor H4	x	
AHSG	α-2-HS-glycoprotein	x	
Ligand binding			
TMOD2	Tropomodulin 2		x
IGFBP1	IGF binding protein 1	x	
MT1X	Metallothionein 1X	x	
CRP	C-reactive protein	x	
APOA2	Apolipoprotein A-II	x	
Signal transduction—other			
BIKE	BMP-2 inducible kinase		x
SGK2	Serum/glucocorticoid reg. kinase 2	x	x
SEL1L	Suppressor of lin-12-like	x	x
SCYE1	Small cytokine E1	x	
ANGPTL3	Angiopoietin-like 3	x	
Signal transduction—receptor			
HAVCR-1	Hepatitis A virus cellular receptor 1		x
TACR3	Tachykinin receptor 3		x
GNB2L1	GTP-binding protein, β2-like 1		x
INSR	Insulin receptor		x
SSTR1	Somatostatin receptor 1	x	x
TM4SF4	Transmembrane 4-4	x	x
ASGR2	Asialoglycoprotein receptor 2	x	
GPR39	G protein–coupled receptor 39	x	
IFNAR1	Interferon receptor 1	x	
TFRC	Transferrin receptor	x	
Transcription Regulation			
ZNF300	Kruppel-like zinc finger protein		x
BCL6	B cell CLL/lymphoma 6		x
ZNF155	Zinc finger protein 155		x
FBX08	F-box only protein 8		x
NR0B2	Small heterodimer protein	x	x
HNF4A7	HNF4α, alternate splice	x	x
NR5A2	LRH-1/FTZ-F1	x	x
ELF3	E74-like factor 3	x	x
NR1D1	THRA1	x	
ATF2	Activating transcription factor 2	x	
CREBL2	CREB-like 2	x	
RARB	RAR-β	x	
Transporter—channel/pore			
SLC17A2	Vesicular glutamate transporter	x	
AQP3	Aquaporin 3	x	
SLC22A11	hOAT4	x	
GJB1	Gap junction protein, β 1	x	
Transporter—lipids and small molecules			
APOH	Apolipoprotein H	x	x
ALB	Albumin	x	
ABCC2	Canalicular OAT	x	
G6PT1	Glucose-6-phosphatase, transport	x	
Transporter—proteins			
RAB6KIFL	RAB6 interacting, kinesin-like		x
PEX13	Peroxisome biogenesis factor 13		x
TMP21	Transmembrane trafficking protein		x
RAB33B	RAS oncogene	x	x
NAPA	α-SNAP	x	
AP3M1	Adaptor-related protein complex	x	
SNX17	Sorting nexin 17	x	

cupied by HNF1α, HNF4α, and HNF6, and the extent to which they are co-occupied by the HNF regulators, differed substantially between these two tissues. For example, the *HNF4-A* gene has two different promoters, which are differentially utilized in hepatocytes and islets (*23*, *24*), and which are differentially occupied by HNF1α, HNF6, and HNF4α in human tissues (Fig. 2A). Because both promoters are used in hepatocytes but only the promoter for HNF4α7 is used in pancreatic islets, it is possible that defects in the regulatory network disproportionately affect HNF4α expression in islets.

The transcription factor binding data were used to identify regulatory network motifs, simple units of transcriptional regulatory network architecture that suggest mechanistic models (Fig. 2B) (*4*, *25*). Our data confirm previous reports that HNF1α and HNF4α occupy one another's promoters in both hepatocytes and islets, forming a multicomponent loop (*23*, *24*, *26*). Multicomponent loops provide the capacity for feedback control and produce bistable systems that can switch between two alternate states (*25*), and it has been suggested that the multicomponent loop present between HNF1α and HNF4α is responsible for stabilization of the terminal phenotype in pancreatic beta cells (*26*). We also found that HNF6 serves as a master regulator for feedforward motifs in hepatocytes and pancreatic islets involving more than 80 genes in each tissue (tables S9 and S11). For example, in hepatocytes, HNF6 binds the promoter for HNF4α7, and HNF6 and HNF4α together bind *PCK1*, which encodes phosphoenolpyruvate carboxykinase, an enzyme key to gluconeogenesis (Fig. 2B). A feedforward loop can act as a switch designed to be sensitive to sustained inputs rather than transient inputs (*25*). HNF1α, HNF4α, and HNF6 were also found to form multi-input motifs by collectively binding to sets of genes in hepatocytes and islets. This regulatory motif suggests coordination of gene expression through multiple input signals. We also found that HNF6, HNF4α, and HNF1α form a regulator chain motif with THRA (NR1D1); regulator chain motifs represent the simplest circuit logic for ordering transcriptional events in a temporal sequence (*4*, *25*). Additional examples of these regulatory motifs can be found in tables S9 to S12 (*20*).

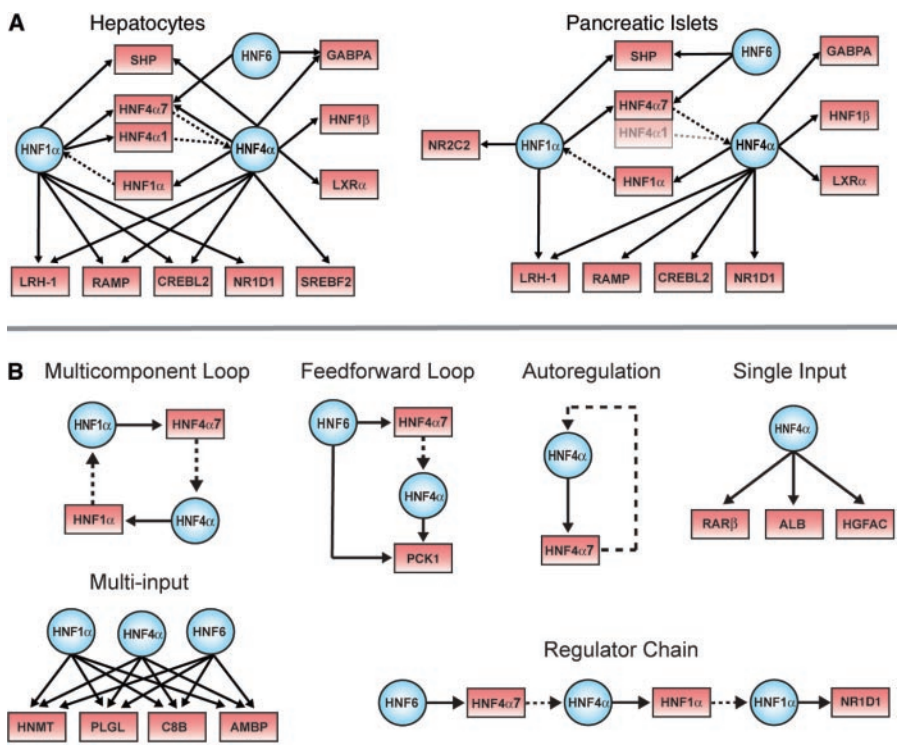

Fig. 2. Transcriptional regulatory networks and motifs. (**A**) HNF1α, HNF6, and HNF4α are at the center of tissue-specific transcriptional regulatory networks. In these examples selected for illustration, regulatory proteins and their gene targets are represented as blue circles and red boxes, respectively. Solid arrows indicate protein-DNA interactions, and genes encoding regulators are linked to their protein products by dashed lines. The HNF4α1 promoter is poorly expressed in pancreatic islets and is thus shaded to reflect this. The HNF4α7 promoter, also known as the P2 promoter (*23*, *24*), is the predominant promoter in pancreatic islets and was recently implicated as a major human diabetes susceptibility locus. For clarity, some gene promoters have been designated by the names of their protein products (e.g., HNF1α for *TCF1*, SHP for *NR0B2*, HNF4α7 for *HNF4A P2*, and HNF1β for *TCF2*). (**B**) Examples of regulatory network motifs in hepatocytes. For instance, in the multicomponent loop, HNF1α protein binds to the promoter of the HNF4α gene, and the HNF4α protein binds to the promoter of the HNF1α gene. These network motifs were uncovered by searching binding data with various algorithms; details on the algorithms used and a full list of motifs found are available in (*20*).

Our results suggest that the nuclear receptor HNF4α contributes to regulation of a large fraction of the liver and pancreatic islet transcriptomes by binding directly to almost half of the actively transcribed genes. This likely explains why HNF4α is crucial for development and proper function of these tissues (*12*–*18*). Furthermore, our results suggest a mechanistic explanation for the recent discovery that polymorphisms in and near *HNF4A*, including those near the promoter used in islets, can increase the risk of type II diabetes (*27*–*30*). We found that multiple HNF factors bind directly to the P2 promoter in primary healthy human islets. Alterations in the binding sites for these factors could cause misregulation of HNF4α expression and thus its downstream targets, leading to beta cell malfunction and diabetes.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S7
Tables S1 to S14

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