Hnf1 α (MODY3) Controls Tissue-Specific Transcriptional Programs and Exerts Opposed Effects on Cell Growth in Pancreatic Islets and Liver^{∇†}

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Heterozygous *HNFL1A* mutations cause pancreatic-islet β -cell dysfunction and monogenic diabetes (MODY3). *Hnf1 α* is known to regulate numerous hepatic genes, yet knowledge of its function in pancreatic islets is more limited. We now show that *Hnf1a* deficiency in mice leads to highly tissue-specific changes in the expression of genes involved in key functions of both islets and liver. To gain insights into the mechanisms of tissue-specific *Hnf1 α* regulation, we integrated expression studies of *Hnf1a*-deficient mice with identification of direct *Hnf1 α* targets. We demonstrate that *Hnf1 α* can bind in a tissue-selective manner to genes that are expressed only in liver or islets. We also show that *Hnf1 α* is essential only for the transcription of a minor fraction of its direct-target genes. Even among genes that were expressed in both liver and islets, the subset of targets showing functional dependence on *Hnf1 α* was highly tissue specific. This was partly explained by the compensatory occupancy by the paralog *Hnf1 β* at selected genes in *Hnf1a*-deficient liver. In keeping with these findings, the biological consequences of *Hnf1a* deficiency were markedly different in islets and liver. Notably, *Hnf1a* deficiency led to impaired large-T-antigen-induced growth and oncogenesis in β cells yet enhanced proliferation in hepatocytes. Collectively, these findings show that *Hnf1 α* governs broad, highly tissue-specific genetic programs in pancreatic islets and liver and reveal key consequences of *Hnf1a* deficiency relevant to the pathophysiology of monogenic diabetes.

A concerted action of multiple transcription factors is required to determine the specialized functions of adult differentiated cells. In recent years, there has been increasing knowledge of the transcription factors that control the differentiated function of insulin-producing β cells (58, 70). Human genetics has provided an important source of information by uncovering transcription factor genes that are mutated in β -cell-deficient forms of diabetes (40, 58, 71, 73). The most common form is due to mutations in *HNFL1A*, encoding hepatocyte nuclear factor-1 α (also known as *Hnf1 α* , *Tcf1*, or *MODY3*) (73). Although first identified as a liver-specific transcription factor, heterozygous *HNFL1A* mutations in humans cause diabetes due to a progressive impairment of β -cell function (7, 31, 65). *HNFL1A*-deficient patients eventually fail to respond to physi-

ological stimuli, such as glucose, yet often retain a considerable ability to secrete insulin in response to sulfonylureas (48).

Although *Hnf1a*-haploinsufficient mice do not develop diabetes (50), *Hnf1a*-deficient mouse and cell line models have been invaluable for understanding the mechanisms underlying β -cell dysfunction. Reduced nutrient-induced insulin release in *Hnf1a*-deficient models has thus been linked to impaired islet aerobic glucose metabolism (13, 50, 66, 67). This in turn is associated with downregulation of selected genes involved in glucose metabolism, such as *Slc2a2* and *Pklr* (4, 46, 60). However, these are not rate-limiting steps in β -cell glycolysis, and therefore, the molecular defects that cause abnormal glucose metabolism in *Hnf1a*-deficient β cells are currently not fully understood (60).

The progressive β -cell phenotype of *HNFL1A*-deficient patients is consistent with a defect in β -cell growth (16). *Hnf1a*^{-/-} mice have been shown to have small pancreatic islets, but it is not certain if this simply correlates with the markedly reduced size and lean mass of *Hnf1a*^{-/-} mice (50). Moreover, there is no documented reduction of β -cell proliferation (50). Severely reduced β -cell mass is observed in mice expressing a dominant-negative form of *Hnf1 α* (23, 72). However, this phenotype is much more severe than in *Hnf1a*^{-/-} mice and may involve the inhibition of other unknown regulatory functions since overexpression of wild-type *Hnf1 α* causes a comparable phenotype (34). The *Hnf1 α* -dependent *Tmem27*

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gene can positively regulate granule function and β -cell mass (2, 17), although the final impact of defective *Tmem27* expression on islet mass in *Hnf1a*-deficient mice has not been specifically studied. Therefore, several studies suggest that Hnf1 α may regulate β -cell growth, yet this has not been conclusively demonstrated.

An important feature of human *HNF1A* deficiency is that it causes a severe β -cell phenotype but only subtle abnormalities in other tissues where homozygous mutant mice have uncovered essential roles (7, 30, 40, 49). This points to cell-specific functions that differ in their sensitivity to haploinsufficiency. Other observations also suggest that Hnf1 α function differs in a fundamental manner in pancreatic islets and liver. For example, selected Hnf1 α target genes (*Slc2a2*, *Pklr*, and *Hnf4a*) are downregulated in *Hnf1a*-deficient pancreatic islets but not in liver (4, 46, 60). Furthermore, despite data suggesting that *Hnf1a* deficiency causes defective β -cell growth, *HNF1A* mutations paradoxically cause hepatocellular adenomas (3). Little is known, however, concerning the extent to which Hnf1 α function differs in these two tissues and the underlying transcriptional mechanisms.

A current limitation of our understanding of Hnf1 α function is that the full spectrum of the genes that are regulated by Hnf1 α in pancreatic islets remains unknown. Genome-wide expression profiling has been carried out with *Hnf1a*^{-/-} liver, showing that Hnf1 α is involved in the regulation of several important hepatic functions, such as bile acid and cholesterol metabolism (59). A large-scale analysis of promoter occupancy has revealed Hnf1 α binding to a considerable number of genes in human islets and liver, pointing to a much broader role for Hnf1 α than anticipated from candidate gene studies (44). However, the functional significance of Hnf1 α binding is unclear. Previous studies, for example, showed that although Hnf1 α binds to *Slc2a2*, *Pklr*, and *Hnf4a* in hepatocytes, this binding is not essential for their transcription in liver (4, 46), raising the possibility that a significant number of Hnf1 α -binding events are not essential for gene transcription.

To address these gaps in knowledge, we integrated the analysis of mRNA expression profiles in *Hnf1a*^{-/-} pancreatic islets and liver with the computational and experimental identification of direct Hnf1 α targets. We show that Hnf1 α regulates key pleiotropic functions in islets that are likely to be central in the pathophysiology of *HNF1A* deficiency. Furthermore, we demonstrate that Hnf1 α plays highly tissue-specific roles in islets and liver, with opposed effects on glucose metabolism and cell growth. We show that tumorigenesis is severely abrogated in *Hnf1a*-deficient β cells expressing the large T antigen (TAG), thus providing a monogenic model that imparts opposed consequences on diabetes and cancer, supporting a notion implied by recent genetic findings in human polygenic type 2 diabetes (20, 76). Finally, our analysis provides insights into how a single transcription factor regulates markedly different cell-specific genetic programs.

MATERIALS AND METHODS

Cell culture and RNA analysis. Mouse pancreatic islets and hepatocytes were isolated from 4- to 6-week-old *Hnf1a*^{-/-}, *Hnf1a*^{+/-}, and *Hnf1a*^{+/+} C57Bl/6J male littermates as described previously (4, 30, 46). Islets were allowed to recover for 2 days in culture in RPMI containing 11 mM glucose supplemented with 10% fetal calf serum and penicillin-streptomycin (1:100; Invitrogen) at 37°C and 5%

CO₂. Total RNA was extracted from cultured islets and freshly dissected liver by using Trizol (Invitrogen). RNA integrity was verified with a model 2100 Bioanalyzer (Agilent). For quantitative PCR (qPCR), total RNA was reverse transcribed as described previously (4, 46). Gene expression levels were analyzed with SYBR green detection with an iCycler (Bio-Rad), using *Tbp* and *Actb* as internal controls, or with TaqMan low-density array cards (Applied Biosystems). The oligonucleotide sequences for the SYBR green assays were designed to span an intron and are given in Table S6 in the supplemental material.

Expression arrays. Two micrograms of liver RNA or 50 ng of islet RNA was amplified through either one single cycle or two cycles of cDNA synthesis, respectively. Labeled cRNA from biological triplicates, each derived from at least two mice, was hybridized to Affymetrix Mouse Genome 430 2.0 arrays. Expression data were normalized using GCOS software. Significance analysis of microarrays was used to identify differentially expressed genes with a 5% false-detection rate (FDR). For each gene, we selected the single most informative probe, showing the lowest *P* value in *Hnf1a*^{-/-}-versus-wild-type comparisons. For the analysis of volcano plots, expression data were normalized with RMA, and the LIMMA package was used for statistical analysis to identify downregulated genes by using a multiple-test-adjusted *P* value of <0.05.

Functional category analysis. The DAVID functional annotation tool (<http://david.abcc.ncifcrf.gov/>) was used to measure the overrepresentation of annotation terms among abnormally expressed genes. The EASE score, a modified Fisher exact *P* value, was used to identify significant enrichments. We also used gene set enrichment analysis (GSEA) implemented with GSEA-P 2.0 (<http://www.broad.mit.edu/gsea>).

Immunofluorescence. Immunofluorescence in paraffin-embedded tissues was performed as described previously (34), using anti-Ki67 (1:50; BD Pharmingen), anti-Ki67 (1:700; Novacastra), anti-insulin II (1:2,000; Chris Van Schravendijk, Vrije Universiteit Brussel), anti-Hnf4 α (1:1,000; Gerhardt Ryffel, Universitätsklinikum Essen), and anti-simian virus 40 (anti-SV40) TAG (1:200; Santa Cruz) antibodies and secondary antibodies conjugated to Cy2 and Cy3 (1:400; Jackson Laboratory).

Generation of insulinomas and analysis of β -cell growth. We crossed *Hnf1a*^{+/-} and *RipTag* (14) mice to generate *Hnf1a*^{+/+}, *Hnf1a*^{+/-}, and *Hnf1a*^{-/-} *RipTag* littermates. Blood glucose was measured three times a week, starting at 8 weeks of age. Mice were sacrificed and analyzed if blood glucose was repeatedly <50 mg/dl or after 9 months. Visible tumors were measured and bisected. The inner mass was removed and plated at 1×10^4 cells per well. Replicate wells were trypsinized after 7 days for cell counting. Ki67 labeling rates in insulin-positive cells were determined by analyzing three nonconsecutive sections from three different pancreases per genotype.

HNF1 motif genome scan. We scanned the mouse genome (assembly mm7) with known TRANSFAC position weight matrices for HNF1 (HNF1_01 and HNF1_C) and selected predictions with scores above 0.85. We collected the genomic coordinates of each predicted HNF1 binding sequence and of the nearest transcription start site (TSS) found in the 5' and 3' orientations. To identify conserved HNF1 motifs, we used a custom Perl script which mapped all HNF1 motifs in the genome scale human-mouse alignments provided by the UCSC Golden Path distribution. We identified HNF1 motifs that were conserved in precisely aligned mouse and human regions but applied a less stringent threshold (0.7 of the same position weight matrix) in the aligned human sequence. We also selected HNF1 motifs with scores of >0.85 that were not located in the exact aligned sequences but differed by <100 bp in their positions relative to the TSSs of orthologs. All HNF1 motif data are available upon request as a MySQL database.

Chromatin immunoprecipitation (ChIP). Approximately 2,000 islets or 2.10^6 isolated hepatocytes were used per immunoprecipitation as described previously (35, 46), with modifications. Briefly, cells were fixed in 1% formaldehyde and sonicated to an average length of 200 to 1,000 bp. Samples were precleared with protein A/G-Sepharose (1:1) (Amersham) and immunoprecipitated with 2 μ g anti-Hnf1 (SC-8986), anti-Hnf1 α (19), or anti-Hnf1 β (SC-22840); 1 μ g anti-H3K4me2 (Upstate 07-030); or anti-mouse immunoglobulin G overnight at 4°C. Immune complexes were collected by adsorption to protein A+G-Sepharose for 1 h at 4°C. Beads were washed, and immunocomplexes were eluted as described previously (35, 46). DNA was purified with QIAquick PCR purification columns (Qiagen), analyzed by qPCR, and compared to a standard curve generated with serial dilutions of input chromatin DNA. The enrichment of target genes was calculated using *Tbp* and *Actb* promoters as a reference. The oligonucleotide sequences are available in Table S6 in the supplemental material.

The HNF1 antibody used for ChIP experiments cross-reacts with Hnf1 β , although in our studies, it is expected to detect only Hnf1 α binding, given that (i) Hnf1 β expression in adult wild-type hepatocytes is insufficient to elicit detectable binding using an Hnf1 β -specific antibody (see Fig. 6) (29) and (ii) gene-specific

assays confirmed comparable enrichment patterns with an Hnf1 α -specific antibody (see Fig. 2B).

ChIP-chip. ChIP and input DNA from three independent experiments were amplified by ligation-mediated PCR and fluorescently labeled with Bioprime (Invitrogen). Five micrograms of labeled DNA was hybridized to Mouse PromoterChip BCBC-5A, containing 1- to 2-kb PCR product tiles from the 5' flanking regions of >12,000 well-characterized genes. Microarray signals were normalized by locally weighted scatter plot smoothing. Statistical analysis of the enrichment ratios (ChIP/input) was performed using eBayes analysis with the LIMMA package. Enriched promoter sequences were defined by a log₂ enrichment value (M) of >0.8 ($P < 0.001$). This stringent threshold was based on a high specificity determined by gene-specific qPCR experiments (see Fig. 2B). It should be noted that lower M thresholds also contain true binding events and are weakly enriched in Hnf1 α -dependent genes (see Fig. S7B in the supplemental material). To integrate expression and binding data, transcripts in Affymetrix Mouse Genome 430 2.0 arrays were matched to genes in BCBC5A promoter arrays on the basis of identical RefSeq or official mouse symbols. Because binding is largely restricted to promoters of genes that are expressed in a particular tissue, the analyses were restricted to genes with present calls. All analyses refer to nonredundant gene sets. An analogous approach was used for matching HNF1 motifs to genes in the Affymetrix expression arrays.

Statistical analysis. An unpaired two-tailed Student *t* test was performed for comparison of gene-specific expression and ChIP enrichments. A chi-square test was used to compare proportions between independent gene groups.

Microarray data accession numbers. Data sets are available in Array Express under accession numbers E-MEXP-1707, E-MEXP-1709, E-MEXP-1714, E-MEXP-1715, and E-MEXP-1979.

RESULTS

Hnf1 α regulates tissue-specific programs in islet cells and liver. HNF1A deficiency in humans primarily results in β -cell dysfunction, yet Hnf1 α is expressed in several epithelial organs, such as the liver, pancreas, intestine, and kidney (7, 8, 49). We have used oligonucleotide arrays to carry out an unbiased analysis of Hnf1 α -dependent gene expression in pancreatic islets and related it to that in liver.

At 4 to 6 weeks of age, Hnf1 α ^{-/-} mice exhibit stunted growth, mild hyperglycemia, renal glycosuria, and liver dysfunction (30, 49). To mitigate the impact of the in vivo milieu of Hnf1 α ^{-/-} mice on islet gene expression, we isolated pancreatic islets from young wild-type and Hnf1 α ^{-/-} mice and placed them in culture for 48 h prior to RNA extraction. Using a 5% FDR, we found 853 downregulated genes and 1,377 upregulated genes in Hnf1 α ^{-/-} islets, representing 5.6% and 9% of all expressed genes, respectively (Fig. 1A; also see Tables S1 and S2 in the supplemental material). Similar values were obtained for Hnf1 α ^{-/-} liver (Fig. 1A; also see Tables S3 and S4 in the supplemental material). A list of genes with >2-fold-decreased expression levels in Hnf1 α ^{-/-} islets is provided in Table 1.

This analysis confirmed previously reported Hnf1 α -dependent islet genes, including *Slc2a2*, *Pklr*, *Tmem27*, *Hnf4a*, *Hnf4g*, and *Foxa3* (2, 4, 17, 60) (Table 1). Differential expression was confirmed by qPCR for 64/70 genes (see Fig. S1A in the supplemental material). Furthermore, 54/59 genes differentially expressed in cultured Hnf1 α ^{-/-} islets were also altered in freshly isolated islets (see Fig. S1B in the supplemental material). The results were not influenced by differences of minor exocrine cell contaminants, since the 13 genes reported to best differentiate between islet and acinar pancreatic tissue (12) did not differ between the two genotypes (not shown).

Gene expression changes in Hnf1 α ^{-/-} islets and liver showed a strikingly limited overlap (Fig. 1A). Thus, only 9.9% of genes downregulated in Hnf1 α ^{-/-} islets were also down-

regulated in liver (Fig. 1A), even though 75% of islet genes and 85% of hepatic genes were coexpressed in both tissues. This indicates that Hnf1 α regulates distinct gene expression programs in pancreatic islets and liver.

As previously reported in studies using Hnf1 α ^{-/-} liver (1, 10, 26, 59), Hnf1 α -dependent genes in both liver and islets predominantly encode diverse metabolic functions (Fig. 1B). Several specific functional categories were enriched among downregulated genes in both tissues, including proteases; membrane transport; and steroid, lipid, and xenobiotic metabolism (Fig. 1B and Table 1). Thus, despite the divergence of Hnf1 α -dependent genes in islets and liver, there are similarities in the functional categories that are positively regulated by Hnf1 α .

In contrast, upregulated genes encoded profoundly different functions in islets and liver. For example, upregulated genes in Hnf1 α -deficient islets were notably enriched in immune response, transforming growth factor β pathway, and mesenchymal marker genes, pointing to an inflammatory response (Fig. 1B). As previously reported, upregulated genes in Hnf1 α ^{-/-} liver were conspicuously enriched in a wide range of metabolic genes, including many involved in lipogenesis and carbohydrate metabolism (1, 26, 59) (Fig. 1B).

Overall, these results indicated that Hnf1 α deficiency affects profoundly different genetic programs in pancreatic islets and liver.

Hnf1 α controls a pleiotropic program in pancreatic islets. A major motivation of these studies was to understand the genetic mechanisms underlying β -cell dysfunction in human HNF1A deficiency. It is immediately apparent that this cannot be ascribed to a single biological process. For a plethora of genes that are severely downregulated in Hnf1 α ^{-/-} islets, there is experimental evidence showing that they exert important regulatory functions on their own in pancreatic islets (Table 1). These genes include *Slc2a2* (21), *Pfkfb2* (39), *G6pc2* (6), *Ddc* (55), *Hnf4a* (22, 47), *Mxipl* (ChREBP) (68), *Tmem27* (2, 17), *Ttr* (52), *Vldlr* (54), and several regulators of β -cell growth (see below). Over 20 transcriptional regulator genes showed >2-fold downregulation (Table 1), indicating a potential for widespread indirect perturbations of gene expression.

One of the most remarkable functional classes comprised the genes regulating nutrient metabolism, a central process in β -cell stimulus-secretion coupling that has been shown to be defective in Hnf1 α -deficient islets (13, 50, 67). In contrast to Hnf1 α -deficient liver, which showed increased expression levels of genes regulating glycolysis and gluconeogenesis (Fig. 1C), Hnf1 α ^{-/-} islets showed decreased expression levels of a strikingly large number of genes of the glycolytic pathway, the tricarboxylic acid (TCA) cycle, and mitochondrial oxidative phosphorylation (Fig. 1C and Table 1; see also Fig. S2 in the supplemental material). In addition to known Hnf1 α targets, several genes involved in the generation and degradation of fructose 2,6-bisphosphate (*Pfkfb2*, *Pfkfb2*, *Fbp1*, and *Fbp2*) were downregulated. The group of downregulated TCA cycle genes included malic enzyme (*Me3*) and fumarate hydratase (*Fh1*) genes. Furthermore, Hnf1 α ^{-/-} islets showed decreased expression levels of numerous genes involved in the metabolism of amino acids (Table 1). These results point to an unexpectedly widespread perturbation of genes required for nutrient metabolism underlying abnormal stimulus-secretion coupling in Hnf1 α ^{-/-} islets.

FIG. 1. *Hnf1a* deficiency causes tissue-specific gene expression changes in pancreatic islets and liver. (A) Pie charts depict the percentages of genes that are downregulated (green) and upregulated (red) in *Hnf1a*^{-/-} pancreatic islets and liver at a 5% FDR. Venn diagrams show the overlap of genes downregulated and upregulated in both tissues. (B) Heat map displaying major functional categories that are enriched among downregulated (green arrows) and upregulated (red arrows) genes in *Hnf1a*^{-/-} islets and liver. (C) GSEA of genes involved in glycolysis and gluconeogenesis, the TCA cycle, and the electron transport chain across genes ranked according to their differential expression levels in *Hnf1a*^{-/-} pancreatic islets (top) and liver (bottom). Vertical lines beneath the graphs depict the rank positions of each gene in the color-coded gene sets. The results show that the three gene sets are downregulated in *Hnf1a*^{-/-} islets yet upregulated in *Hnf1a*^{-/-} liver. WT, wild type; KO, knockout.

Another richly represented gene set comprised genes encoding regulatory proteases and inhibitors of proteases (Table 1). Many were not known to be expressed in pancreatic islets, and their relevant islet substrates are thus unknown. For others, a relation to the *Hnf1a*-deficient phenotype can be anticipated. For example, the direct targets α -1-microglobulin (*Ambp*) and protein C (*Proc*) have known anti-inflammatory roles, and *Proc* has been shown to improve the mass of transplanted islets (11, 28), suggesting a mechanism whereby Hnf1 α may prevent the inflammatory response observed in mutant islets (Fig. 1B). Taken together, these results suggest that β -cell dysfunction in *Hnf1a*-deficient islets results from the integration of broad gene expression defects affecting diverse functions of β cells rather than from the abnormality of a unique pathway.

Identification of genomic targets of Hnf1 α . To gain insights into the mechanisms underlying tissue-specific Hnf1 α -dependent gene regulation, we searched for genomic targets of

Hnf1 α . In one approach, we used liver chromatin and an Hnf1 antibody to hybridize mouse BCBC-5A promoter microarrays. Using a stringent threshold (M values of ≥ 0.8 and P values of < 0.001), we identified Hnf1 α binding to 194 promoters (Fig. 2A). Control experiments with immunoglobulin G in liver showed no binding with these criteria (see Fig. S6 in the supplemental material). We selected 17 genes not previously reported as direct Hnf1 α targets and verified in all cases binding in liver chromatin by single-gene qPCR using the same Hnf1 antibody and using an independent Hnf1 α -specific antibody (Fig. 2B), thus confirming the specificity of the ChIP-chip procedure.

A genome-wide scan reveals conserved HNF1 motifs in the 5' regions of Hnf1 α -dependent genes. To complement the experimental detection of binding in promoter arrays, we performed a computational scan of the entire mouse genome. This identified 634,622 high-affinity HNF1 binding sequence

TABLE 1. Genes downregulated in *Hnf1a*^{-/-} islets

Function(s)	Gene ^a	Fold change	ChIP result ^d		Binding ^e	HNF1 motif ^f
			Islet	Liver		
Glucose metabolism, electron transport, and oxidative phosphorylation	<i>Slc2a2</i> (<i>Glut2</i>) ^b	-55.6	+	+	M	
	<i>Alox12e</i>	-11.6		-		
	<i>Fbp2</i> ^b	-10.8		-		
	<i>Ppp1r1a</i>	-9.4		-	-	-3876/+6658
	<i>Fbp1</i> ^b	-7.9	+	+		-3609
	<i>Pklr</i>	-5.1	+	+	MH	-2318 (MH)
	<i>Cox6a2</i>	-4.4		-		
	<i>Me3</i>	-3.3				
	<i>Fh1</i>	-3.1	+	+	MH	-351
	<i>Pgk1</i>	-2.9		-		
	<i>Pfkfb2</i> ^b	-2.9		-		-2498 (MH)
	<i>Pfkl</i>	-2.7		-		
	<i>Pdk1</i>	-2.5		-		
	<i>G6pc2</i> (<i>IGRP</i>) ^b	-2.4		-		-501
	<i>Atp7a</i>	-2.3		-		
	<i>Tpi1</i>	-2.3		-		
	<i>Pfkfb</i>	-2.3		-	-	
	<i>Ndufs2</i>	-2.2		-	-	
	<i>Slc37a4</i> (<i>G6PT</i>)	-2.0		+		-2236
Amino acid transport and metabolism	<i>Cbs</i>	-29.4		-	H	
	<i>Ddc</i> ^b	-12.7		-	H	-816 (MH)
	<i>Dct</i>	-10.6		-		
	<i>Slc38a4</i>	-7.8				-1990 (MH)
	<i>Tat</i>	-5.2				+2734
	<i>Cdo1</i>	-5.1			-	
	<i>Slc12a7</i>	-3.8		-		-2356
	<i>Gatm</i>	-3.4		-	-	+4917
	<i>Glul</i>	-3.3		-	-	
	<i>Ccbl2</i>	-3.1				
Lipid metabolism	<i>Elovl2</i>	-10.3				-2985
	<i>Ptgs2</i>	-4.6		-	-	
Cell adhesion, intercellular communication, and extracellular matrix	<i>Tm4sf4</i> ^c	-13.5	+	+		-151 (MH)
	<i>Muc4</i>	-10.4				
	<i>Cdh8</i>	-7.6		-	-	-3642
	<i>Pkhd1</i>	-7.4	+	+		-80 (MH)
	<i>Pelkc</i> ^c	-7.1				-54 (MH)
	<i>Sema4a</i>	-7.0	+	+	M	-793 (MH)
	<i>Col27a1</i>	-4.3		-		+1069
	<i>Anxa4</i>	-3.6		+		
Cytoskeleton, secretion, vesicle transport, and endoplasmic reticulum and Golgi function			+			
	<i>Kif12</i>	-62.5	+	-	-	-23 (MH)
	<i>Tmed6</i>	-33.3	+	-	H	-53 (MH)
	<i>Golt1a</i>	-25.2		+		-66 (MH)
	<i>Vil1</i>	-12.0		+		
	<i>Anks4b</i> (<i>Harp</i>)	-9.4		+		-35 (MH)
	<i>Mtmr11</i>	-8.9			H	-2045/+389
	<i>Rab37</i>	-6.9		-		-1302
	<i>Tmem27</i> ^{b,c}	-3.4	+	-		-54 (MH)
Iron transport and metabolism	<i>Sfxn2</i>	-7.9		-	-	
	<i>Mfi2</i>	-5.9		-		
	<i>Slc40a1</i>	-5.8		-	MH	+4645
Membrane ion transport	<i>Slco1a6</i>	-18.2				-3471
	<i>Slc26a1</i>	-7.7		+		
	<i>Atp4a</i>	-4.4		-		-3154
	<i>Clic5</i>	-3.9				
	<i>Fxyd3</i>	-3.7		-		
	<i>Trpm2</i>	-3.6				
Proteolysis and peptidolysis	<i>Trpm5</i>	-3.3				-2325
	<i>Cpn1</i>	-76.9	+	+		-53 (MH)
	<i>Ambp</i>	-55.6	+	+	MH	-3017 (MH)
	<i>Hgfac</i> ^c	-50.0	+	+	M	-95 (MH)
	<i>Serpina10</i>	-50.0		+		-108 (MH)
	<i>Dpp4</i>	-41.7		-		-311/+6912
	<i>Tmprss4</i> ^c	-33.3		-		
	<i>Rnf186</i>	-12.8		+	MH	-133 (MH)
	<i>Serpina7</i>	-11.6				
	<i>Tmprss2</i>	-10.0		-		

Continued on following page

TABLE 1—Continued

Function(s)	Gene ^a	Fold change	ChIP result ^d		Binding ^e	HNF1 motif ^f
			Islet	Liver		
	<i>Adam32</i>	−7.9		−		−455
	<i>Pcsk9</i>	−7.9	+	+	MH	+141
	<i>Ramp</i>	−6.1		−		−3356/+1383
	<i>Ace2</i>	−5.8				−67 (MH)
	<i>Cpb2</i>	−4.7		−		−900/+1335
	<i>Enpep^c</i>	−4.5		+		
	<i>Proc^b</i>	−3.8		−		−20 (MH)
	<i>Pcsk1^b</i>	−2.1		−	−	−2008
Hormone transport and metabolism	<i>Gc (VDBP)</i>	−32.3	+	+		−186 (MH)
	<i>Trp^b</i>	−14.5	+	+	MH	−39 (MH)
Membrane receptors and ligands	<i>Prly^{b,c}</i>	−15.2		−		−838
	<i>Nptx2</i>	−14.1		−		−1917 (MH)/+1392
	<i>Pigr</i>	−10.6		−		+5897
	<i>Tacr3</i>	−10.4			−	−2182 (MH)
	<i>Ccl28</i>	−7.5				
	<i>Gpr109a</i>	−7.0		−		−371
	<i>Rnase4/Ang1^c</i>	−5.9	+	+		−172 (MH)
	<i>Gpr137b</i>	−4.3		−		+887
	<i>Vldlr^b</i>	−4.2				−2244
	<i>Ffar2</i>	−4.1				
	<i>Igf1r^{b,c}</i>	−2.7				−3729
	<i>Glp1r^{b,c}</i>	−2.7				−865
Kinases and intracellular signaling	<i>Sgk2</i>	−12.7	+	+		−152 (MH)
	<i>Mapk15 (ERK7)</i>	−8.2				+2961
	<i>Wnk4</i>	−6.6				+2908
	<i>Grtp1</i>	−4.9		−		
	<i>Frk (Bsk)^{b,c}</i>	−4.3			H	
	<i>Gng12</i>	−3.2		−	−	−1708
Transcriptional regulation	<i>Pou3f4</i>	−9.5		−		−507
	<i>Hnf4a^{b,c}</i>	−8.8	+	+	MH	−100 (MH)
	<i>Fbxl10</i>	−7.1				+5979
	<i>Foxa3</i>	−6.9	+	+	−	−1794
	<i>Bcl6b</i>	−5.5			−	
	<i>Msh5</i>	−5.4		+	MH	−490 (MH)
	<i>Nr1h4 (Fxr)^c</i>	−5.1	+	+	MH	−72 (MH)
	<i>Etv5^c</i>	−4.9			−	−2325
	<i>C030010B13Rik</i>	−4.4				−1865
	<i>Hod</i>	−4.1				
	<i>Mxipl (ChREBP)^b</i>	−3.4				
Xenobiotic metabolism, detoxification, and sulfotransferases	<i>Ugt2b34</i>	−125.0		+		−1387
	<i>Sult1d1</i>	−31.3	+	+		−127
	<i>Akr1c19</i>	−12.3				−41
	<i>Sult1c2</i>	−8.3				−522
	<i>Ugt1a1</i>	−7.9			MH	−34 (MH)
	<i>Ugt2b35</i>	−5.4		+		−1725
	<i>Adh1</i>	−4.6		+		−1874
	<i>Akr1c13</i>	−3.6				−135
	<i>Cyp4v3</i>	−3.4		+	−	−1203
	<i>Akr1c12</i>	−3.4		+		−99

^a All genes having known functions and downregulated >3-fold in *Hnf1a*^{−/−} islets and selected genes regulated <3-fold are listed.

^b This gene has a known function in pancreatic islets (see citations in the text).

^c This gene has a known function in cell proliferation or tumorigenesis (see citations in the text and in Table S5 in the supplemental material).

^d “−” indicates a gene represented in the array but not enriched. Islet, Hnf1α binding in islets (ChIP-qPCR); liver, Hnf1α binding in hepatocytes (ChIP-chip and ChIP-qPCR).

^e Results for a systematic species comparison of binding (43) are shown, with Hnf1α binding in hepatocytes in mouse only (M), human only (H), or mouse and human (MH) or not bound (−).

^f Each value represents the position of the closest computational HNF1 motif relative to the TSS of the murine gene. “MH” indicates that this motif is conserved in humans.

motifs (63). Of these, 2.3% were within 5 kb of the TSSs of genes, 36.0% were intragenic, and 59.7% were intergenic. Because not all such sequences are expected to be bona fide binding sites, we focused on motifs that were evolutionary conserved in the mouse and human genomes.

HNF1 motifs exhibited a prominent depletion in the proximity of the TSS (Fig. 3A). This was plausibly because of the sequence bias of 5′ flanking regions, as it was observed for

other motifs with AT content similar to that of HNF1 but not with GC-rich motifs (see Fig. S3 in the supplemental material). Remarkably, evolutionary conserved HNF1 motifs displayed an inverse pattern, with a distinct peak in the 200-bp-upstream regions of genes (Fig. 3A). Proximal conserved HNF1 motifs were enriched 10- and 20-fold among genes that were downregulated in *Hnf1a*^{−/−} islets and liver, respectively ($P < 0.0001$), whereas this was not observed in upregulated genes

FIG. 2. Hnf1 α -binding analysis in hepatocytes and pancreatic islets. (A) M-A plots of Hnf1 ChIP hybridization intensities in hepatocytes. Dark dots depict Hnf1-bound genes ($M > 0.8$ and $P < 0.001$). (B) Validation of 25 Hnf1 targets in hepatocytes and islets by qPCR. Hnf1 targets in hepatocytes were validated using the Hnf1 antibody and an Hnf1 α -specific antibody. Consistent with observations that Hnf1 β was not detected in adult hepatocytes (29) (Fig. 6), similar results were obtained with the two antibodies. Gene-specific qPCR signals were calculated as percentages of input DNA and expressed as the enrichment values relative to the mean values for *Tbp* and *Actb*. Seven genes were tested due to the presence of a proximal conserved HNF1 motif and are marked with a circle. Genes are grouped according to their tissue expression patterns.

(Fig. 3B and C). No comparable enrichment of HNF1 motifs in Hnf1 α -dependent genes was observed in other gene locations, even when the entire intragenic or 3' regions were computed as single areas (see Fig. S4 in the supplemental material). Thus, an unbiased genome-wide scan revealed that evolutionary conserved HNF1 binding sequences are strongly enriched in the 5' regions of Hnf1 α -dependent genes.

Hnf1 α binding correlates with computational target prediction. Among Hnf1 α -bound regions, 36% contained an HNF1 motif (a 5.5-fold enrichment over unbound genes; $P < 0.0001$), 60% of which were evolutionary conserved (Fig. 3D; see also Fig. S7A in the supplemental material). The overlap between predicted and observed binding levels was highest among genes that were downregulated in *Hnf1 α ^{-/-}* liver or islets (Fig. 3D) or when motifs were located in the immediate 5' region (Fig. 3E). These findings point to a highly significant yet partial overlap of computationally predicted and experimentally determined Hnf1 α -binding sites. Most importantly, they show that proximal, evolutionary conserved HNF1 motifs in Hnf1 α -dependent genes are highly predictive of direct Hnf1 α targets.

Shared and tissue-specific Hnf1 α binding in islets and liver. We next compared Hnf1 α binding to genomic targets in liver and islet chromatin. All genes that were bound by Hnf1 α in liver in promoter arrays and were expressed in islets were also bound in islets in single-gene qPCR assays (Fig. 2B). However, none of the tested promoters of genes expressed in liver but not islets showed binding in islets (Fig. 2B). Similarly, genes with predicted HNF1 binding sequences that were expressed in islets but not liver were bound almost exclusively in islets (Fig. 2B). To confirm that Hnf1 α binding in pancreatic islets occurred in β cells, we reproduced binding in 17/18 genes in Min6 β cells (see Fig. S5 in the supplemental material). Thus, Hnf1 α binding in 5' proximal regions is frequently shared in islets and liver, except for genes that are selectively expressed in either tissue, in which case binding is specific for that tissue.

Hnf1 α is an essential activator for a subset of its targets. To understand how Hnf1 α exerts its regulatory function through

its direct targets, we integrated gene expression and genomic target datasets. Unexpectedly, only ~15% of genes with either experimental or predicted Hnf1 α binding exhibited changes in mRNA in *Hnf1 α ^{-/-}* liver or islets (Fig. 4A and B). These results could be influenced in part by the use of different platforms for expression and binding analysis or due to mRNA levels being not exclusively dependent on gene transcription. We thus compared levels of histone H3 lysine 4 dimethylation (H3K4me2), an active chromatin mark, in *Hnf1 α ^{-/-}* and control liver by using BCBC promoter arrays. This experiment showed that only some Hnf1 α targets exhibit Hnf1 α dependence to maintain an active chromatin configuration (Fig. 4C). Thus, Hnf1 α is not essential for the activity of a sizeable fraction of its target genes.

Hnf1 α -bound and predicted targets were nevertheless strongly enriched among genes with decreased mRNA and H3K4me2 in *Hnf1 α ^{-/-}* islets and liver, particularly among the genes that were most profoundly perturbed (GSEA $P < 0.001$) (Fig. 4C and D; see also Fig. S7B in the supplemental material). In contrast, Hnf1 α binding was not enriched among up-regulated genes, suggesting that the latter largely reflect indirect cellular responses or a repressive function of Hnf1 α that does not require a direct interaction with DNA.

Interestingly, genes that were bound by Hnf1 α and showed decreased expression in *Hnf1 α ^{-/-}* islets were as a group also downregulated in *Hnf1 α ^{+/-}* islets (GSEA $P < 0.001$) (Fig. 5). Thus, although *Hnf1 α ^{+/-}* mice are not diabetic, they reveal that the direct functions of Hnf1 α in islets are sensitive to gene dosage.

These results therefore suggest that Hnf1 α preferentially acts as a transcriptional activator in islets and liver, where it is essential for achievement or maintenance of an active gene state only in a subset of its targets, and that haploinsufficiency impairs this function.

The Hnf1 α dependence of direct targets is tissue specific. We next assessed if tissue-specific *Hnf1 α* dependence patterns, rather than only tissue-specific Hnf1 α binding, could underlie

FIG. 3. Conserved HNF1 motifs are selectively enriched in the immediate 5' flanking regions of genes downregulated in *Hnf1a*^{-/-} islets and liver. (A) Frequency of HNF1 motifs in 100-bp windows relative to the TSSs of all RefSeq genes. We scanned all HNF1 motifs in the mouse genome (yellow line) and conserved motifs in mouse and human aligned genomes (blue line). (B) Frequency of conserved HNF1 motifs in 200-bp windows relative to the TSSs of genes that were downregulated and upregulated in *Hnf1a*^{-/-} islets (left) and liver (right). (C) Percentages of genes that were downregulated in *Hnf1a*^{-/-} islets and liver among genes containing nonconserved and conserved HNF1 motifs within 200 bp upstream of the TSS. The analysis was restricted to genes that are expressed in each tissue (*, *P* values of <0.001; **, *P* values of <0.0001 for comparison to genes that lack an HNF1 motif within 200 bp upstream of the TSS). (D) Venn diagram showing the overlap between Hnf1α-bound genes in ChIP-chip (red) and genes with an HNF1 motif (blue) or a conserved HNF1 motif (green) within 1 kb upstream of the TSS. For the right panel, the same analysis was conducted for genes that were downregulated >2-fold in *Hnf1a*^{-/-} liver or islets. Both analyses were restricted to expressed genes represented in the promoter arrays (*n* = 8,803). (E) Frequencies of Hnf1α binding to genes with nonconserved and conserved HNF1 motifs at different distances from the TSS. The dashed line represents the overall percentage for all RefSeq genes expressed in liver (*, *P* values of <0.05; **, *P* values of <0.001; ***, *P* values of <0.0001 for comparison to the overall Hnf1α-binding frequency).

the different transcriptome phenotypes in islets and liver. We thus compared the behavior patterns of direct targets in pancreatic islets and liver. This revealed that the subset of direct Hnf1α targets that were downregulated in *Hnf1a*^{-/-} mice were in most cases selectively downregulated in either islets or liver (Fig. 6A).

Interestingly, we observed that Hnf1α targets expressed in both tissues were 3.1- to 5.2-fold more frequently downregulated in *Hnf1a*^{-/-} islets than in liver (Fig. 6B). Gene-specific qPCR assays with liver confirmed a lack of gene expression perturbation in a set of eight Hnf1α targets that were severely downregulated in pancreatic islets (see Fig. S1C in the supplemental material). When considering gene expression changes in the liver, we observed that Hnf1α-targets expressed selectively in liver were downregulated in *Hnf1a*^{-/-} mice in 41 to 58% of cases, in marked contrast to those that were expressed in islets and liver, which were downregulated in 10% of cases (Fig. 6B). Thus, in pancreatic islets, Hnf1α is frequently essential for genes that are expressed in both islets and liver,

whereas direct essential functions of Hnf1α in liver largely occur in liver-selective genes (Fig. 6C).

Hnf1β occupies target genes that do not show Hnf1α dependence. Hnf1β is a paralog of Hnf1α with indistinguishable *in vitro* DNA-binding properties (8). The expression of Hnf1β in adult hepatocytes is very low or undetectable but has previously been shown to be increased in *Hnf1a*^{-/-} liver (9, 49). In adult pancreas, Hnf1β is expressed in ductal cells but not in wild-type or *Hnf1a*^{-/-} islet cells (37, 41, 56) (see Fig. S9 in the supplemental material).

ChIP studies with Hnf1β-specific antisera showed either no binding or very low levels of binding in wild-type hepatocytes, consistent with its low abundance (Fig. 6D). In *Hnf1a*^{-/-} hepatocytes, where Hnf1β expression is induced, Hnf1β occupancy was elicited selectively in Hnf1α targets whose expression or H3K4me2 state was not affected by *Hnf1a* deficiency but not in genes that exhibited Hnf1α-dependent gene activity (Fig. 6D). As expected, Hnf1β did not bind any gene in wild-type or *Hnf1a*^{-/-} islets (results not shown). This suggests that in hepa-

FIG. 4. Only a subset of Hnf1 α -bound genes are affected by *Hnf1a* deficiency in islets and liver. (A, B) Volcano plot relating the change and statistical significance of gene expression in *Hnf1a*^{-/-} (KO) versus wild-type (WT) liver (A) and islets (B). All genes are represented with gray dots. Colored dots are direct Hnf1 α targets identified by either ChIP-chip (red) or a proximal conserved HNF1 motif (green). (C) Volcano plot displaying differential H3K4me2 levels in gene promoters in *Hnf1a*^{-/-} and wild-type hepatocytes. Red dots depict direct Hnf1 α targets as in panel A. (D) Percentage of direct Hnf1 α targets as a function of the magnitude of gene expression changes in *Hnf1a*^{-/-} islets. "NC" represents all genes lacking differential expression. Direct Hnf1 α target genes were defined by Hnf1 α binding in ChIP-chip (red), a conserved HNF1 motif (green), or either feature (blue).

FIG. 5. Functional Hnf1 α target genes are downregulated in islets from *Hnf1a*-haploinsufficient mice. (A) Scatter plot of gene expression changes in *Hnf1a*^{+/-} (log₂ heterozygous mutant [HET]/wild-type [WT] values) and *Hnf1a*^{-/-} (log₂ knockout [KO]/WT values) islets. Dark spots represent direct Hnf1 α targets (identified by either ChIP-chip or proximal conserved HNF1 motifs) that were downregulated >2-fold in *Hnf1a*^{-/-} islets. (B) GSEA of Hnf1 α -bound genes that were downregulated >2-fold in *Hnf1a*^{+/-} islets across genes ranked according to their differential expression levels in *Hnf1a*^{+/-} pancreatic islets. The vertical lines beneath the graphs depict the rank positions of the genes.

cytes, Hnf1 β exerts a redundant regulatory role in a subset of direct Hnf1 α targets that are also expressed in pancreatic islets, while Hnf1 β cannot compensate for Hnf1 α function in liver-selective targets or in pancreatic islets. Thus, Hnf1 α can play different essential roles among direct genes that are expressed in both pancreatic islets and liver, and this is likely to partly reflect a gene-selective compensatory function of Hnf1 β in *Hnf1a*^{-/-} liver.

Hnf1 α exerts opposed effects on cell growth in pancreatic islets and liver. We next examined possible biological consequences of the markedly different Hnf1 α -dependent transcriptional changes in islets and liver. One of the most striking contrasts was the in expression levels of genes regulating cell growth (Fig. 7A and B). Most notably, among Hnf1 α -dependent genes in islets, we noted decreased expression levels of many genes with known mitogenic and survival functions in β cells, including receptors (*Prlr*, *Glp1r*, and *Igf1r*) and activators (*Hgfa*) of growth factors (18, 64) (Table 1 and Fig. 7A), suggesting that Hnf1 α may be a major regulator of β -cell growth.

As previously reported, Ki67 and BRDU labeling indexes were similarly low in adult *Hnf1a*^{-/-} and control islets (50) (not shown). We nevertheless observed a moderate reduction in Ki67 labeling rates in neonatal *Hnf1a*^{-/-} β cells (6.9% versus 5.0%; $P = 0.03$). Also consistent with previous studies, islet size in young *Hnf1a*^{-/-} mice was decreased (50), although the number of extra-islet β cells was increased and the overall relative pancreatic area occupied by β cells was not markedly decreased (38) (not shown). Interpretation of β -cell mass in *Hnf1a*^{-/-} mice is confounded by their in vivo status, including the presence of markedly lower lean mass, liver dysfunction, and diabetes (30, 50). Experimental models of β -cell growth, such as pregnancy and high-fat feeding, are not feasible for *Hnf1a*^{-/-} mice, because of their infertility, fatty liver, and high mortality rate (30, 50). We thus tested β -cell growth in *Hnf1a*^{-/-} islet cells bearing the *RipTag* transgene, which uses

FIG. 6. *Hnf1α* requirement for expression of its direct targets is tissue specific. (A) Venn diagrams showing all *Hnf1α* direct targets (black ovals) and the subsets that are downregulated >2-fold in *Hnf1α*^{-/-} islets (left circle in each oval) and liver (right circle in each oval). Separate analyses were performed for genes containing conserved HNF1 motifs 200 bp upstream of the TSS and those bound in ChIP-chip. (B) Percentages of direct *Hnf1α* target genes that were downregulated >2-fold in *Hnf1α*^{-/-} islets or liver, broken down according to their tissue expression patterns: IL, expression in both islets and liver; I, expression only in islets; L, expression only in liver. (C) Expression patterns for all direct targets that are downregulated >2-fold in *Hnf1α*^{-/-} mouse tissues. (D) Enrichment of Hnf1β and H3K4me2 in HNF1 targets in wild-type (WT) and *Hnf1α*^{-/-} (KO) hepatocytes. In *Hnf1α*^{-/-} hepatocytes, Hnf1β binds to all tested *Hnf1α* targets that are not silenced in *Hnf1α*^{-/-} liver but not to those that become inactive. Genes are grouped according to their tissue expression patterns.

the insulin promoter to direct expression of the SV40 large TAg oncogene (14). The TAg showed a robust early expression in both *Hnf1α*^{+/+} and *Hnf1α*^{-/-} *RipTAg* β cells (see Fig. S8 in the supplemental material). As expected from previous experiments using this transgene in diverse genetic backgrounds (14), at 3 months of age, large insulinomas were invariably formed in *Hnf1α*^{+/+} *RipTAg* mice (Fig. 7C). In contrast, *Hnf1α*^{-/-} *RipTAg* mice developed small hyperplastic islets, and only 2 of 20 mice formed small insulinomas at 9 months of age (Fig. 7C). We used Ki67 colabeling with insulin to assess β-cell proliferation and found the value to be reduced to 43% of normal values in β cells from *Hnf1α*^{-/-} *RipTAg* versus *Hnf1α*^{+/+} *RipTAg* mice (Fig. 7D and F). Similarly, Ki67 colabeling with TAg was decreased to 54% of normal values in *Hnf1α*^{-/-} *RipTAg* mice (not shown). Furthermore, β cells from *Hnf1α*^{-/-} *RipTAg* mice failed to grow in culture (Fig. 7E). Thus, defective tumor formation was at least in part due to decreased proliferation of TAg-positive (TAg⁺) β cells in vivo and in vitro (Fig. 7D, E, and F).

Hnf1α-deficient mice develop hepatomegaly and steatosis,

and humans with MODY3 frequently exhibit hepatocellular adenomas (3). *Hnf1α*^{-/-} hepatocytes showed increased expression levels of multiple genes involved in mitosis and cell cycle regulation, including those encoding several cyclins and Ki67 (Fig. 7B). This prompted us to examine hepatocyte proliferation. Remarkably, the frequency of Ki67⁺ hepatocytes increased fivefold in *Hnf1α*^{-/-} liver (Fig. 7G and H). Thus, *Hnf1α* regulates genes required for cell proliferation and is required for TAg-induced tumorigenesis in pancreatic islets yet suppresses cell growth in liver.

DISCUSSION

***Hnf1α* controls a pleiotropic islet cell program.** We have provided for the first time an unbiased large-scale assessment of *Hnf1α*-dependent gene expression in pancreatic islets. Our results confirm previously reported candidate gene studies (2, 4, 17, 46, 59, 60, 66) and significantly expand the list of *Hnf1α*-dependent islet genes. A major conclusion of this analysis is that the altered profile of *Hnf1α*^{-/-} islets is markedly pleio-

FIG. 7. *Hnf1α* deficiency has opposed effects on β-cell and hepatocyte proliferation. (A, B) Heat maps of expression changes (represented as log₂ *n*-fold change) in *Hnf1α*^{−/−} islets and liver for selected genes involved in cell growth, survival, and tumorigenesis (A) or cell cycle progression (B). (C) Low-magnification view of hematoxylin-and-eosin (H&E) staining of pancreases from *Hnf1α*^{+/+} *RipTag* and *Hnf1α*^{−/−} *RipTag* mice. Dotted lines depict normal islets (i), hyperplastic islets (h), and fully developed insulinoma tumors (t). At 3 months of age, insulinomas were invariably identified in *Hnf1α*^{+/+} *RipTag* mice but never in *Hnf1α*^{−/−} *RipTag* mice. (D) Percentages of Ki67⁺ β cells in *Hnf1α*^{+/+} and *Hnf1α*^{−/−} *RipTag* mice. wt, wild type; ko, knockout. (E) Changes in β-cell number after 7-day culture of dispersed insulinoma β cells from 3-month-old *Hnf1α*^{+/+} *RipTag* mice and from a 9-month-old *Hnf1α*^{−/−} *RipTag* mouse that developed small insulinomas. β cells from *Hnf1α*^{−/−} *RipTag* insulinomas failed to grow in culture. (F) Immunostaining of Ki67 (red) and insulin (green) in pancreases from *Hnf1α*^{+/+} *RipTag* and *Hnf1α*^{−/−} *RipTag* mice. (G) Percentages of Ki67⁺ Hnf4α-expressing hepatocytes in 1-month-old control and *Hnf1α*^{−/−} liver samples. (H) Immunostaining of Ki67 (red) and Hnf4α (green) in liver samples from control and *Hnf1α*^{−/−} mice.

tropic, affecting genes spanning a broad spectrum of cellular functions (Table 1). For over a dozen genes that are markedly downregulated in *Hnf1α*^{−/−} islets, there is experimental evidence indicating that they exert regulatory functions on their own in islets (Table 1). β-Cell dysfunction in *HNFLA*-deficient diabetes is therefore likely to result from the failure of a broad cell-specific genetic program rather than a derangement of a discrete biological pathway.

Expression of genes involved in nutrient metabolism in *Hnf1α* deficiency. *HNFLA*-deficient humans and mice exhibit a severe abrogation of glucose- and amino acid-induced insulin secretion (7, 13, 50). This has been linked to defective islet-cell glycolytic flux and oxidative phosphorylation (60, 66, 67). Although selected candidate gene defects have been proposed to be involved (60, 66, 67), we documented profoundly reduced expression in *Hnf1α*^{−/−} islets for over 20 genes involved in

glycolysis, oxidative phosphorylation, and derivation of amino acids to the TCA cycle. Downregulation of two critical TCA cycle enzymes, malic enzyme 3 (*Me3*) and fumarate hydratase (*Fh1*), is likely to contribute to the defective mitochondrial metabolism (36, 67). Decreased expression levels were observed in numerous genes involved in upper glycolysis and gluconeogenesis, including those encoding enzymes with unknown and known functions in β cells, such as the bifunctional *Pfkfb2* enzyme (39) and the glucose-6-phosphatase catalytic subunit-related (*G6pc2*) protein (6). Other *Hnf1 α* -dependent genes are potentially linked to β -cell metabolic oscillations, namely, fructose biphosphatase (*Fbp1* and *Fbp2*) (51) and phosphofructokinase (*Pfkl* and *Pfkp*) genes (69). Finally, the broad defect in the expression of amino acid enzyme genes is also likely to affect nutrient recognition, given the widespread roles of amino acids in β -cell stimulus-secretion coupling (42). Importantly, although numerous nutrient metabolism enzyme genes were likely downregulated due to indirect effects, a substantial number were direct *Hnf1 α* targets (Table 1). In summary, our results are consistent with previous findings of abnormal nutrient metabolism in *Hnf1a* deficiency (13, 60, 66, 67) but indicate that this abnormality most likely results from the integrated deregulation of a large number of genes rather than from one or few gene defects.

***Hnf1 α* exerts opposed roles on cell growth and oncogenesis in islets and liver.** Islet size is reduced in *Hnf1a*^{-/-} mice, but it is unclear if this is inappropriate for the severely diminished lean mass or potentially secondary to the associated metabolic derangement (50). Because baseline proliferation levels are already low for normal β cells, it has so far not been possible to determine if these levels are further impaired by *Hnf1a* deficiency. In the current study, we detected a mild reduction of proliferative β cells in pancreases from embryos and newborn mice and showed that *Hnf1a*^{-/-} β cells expressing the SV40 TAg had unambiguously low levels of *in vivo* and *in vitro* proliferation and a severe impairment in tumor formation.

The observed spectrum of gene defects strongly suggests that the abnormal- β -cell-growth phenotype is multifactorial rather than consequent to the decreased expression of a single target gene. Among several non-mutually exclusive candidate mechanisms, we note defective expression levels of multiple growth factor receptors, ligands, transduction regulators, and regulatory proteases, several of which are direct targets (Table 1; also see Tables S1 and S5 in the supplemental material). Furthermore, abnormal glucose metabolism plays a central role in β -cell growth (62) and is thus expected to be instrumental in this phenotype. Regardless of the precise underlying molecular mechanisms, the demonstration that the *Hnf1 α* -dependent genetic program regulates cell growth and oncogenesis in β cells is important because, despite differences in physiological contexts, it lends support to the notion that impaired growth of β cells may, together with other islet-cell defects, contribute to the development of diabetes that typically occurs in *HNF1A*-deficient patients after the first decade.

This finding is particularly intriguing because it provides a clear monogenic correlate to the recent discovery that genetic variation in several loci, including, for example, *TCF2/HNF1B* and *JAZF1*, exhibits opposed effects on susceptibility for cancer and type 2 diabetes (20, 76). *Hnf1a* deficiency thus represents a clear example whereby a genetic variant that imparts a

deleterious effect on β -cell growth exerts a beneficial effect on tumor formation.

The stimulatory role of *Hnf1 α* in β -cell growth and tumor formation is paradoxical, because humans with heterozygous *HNF1A* mutations frequently develop hepatic adenomas (3). Such tumors result from somatic biallelic loss of function of *HNF1A* (3). Our data now show that, in contrast to that in β cells, *Hnf1a* deficiency in hepatocytes causes increased expression of proliferation and cell cycle regulatory genes. Several direct targets that were downregulated in *Hnf1a*^{-/-} liver are candidate mediators of this phenotype, including *Nr1h4* and *Nr0b2*. Mice lacking the farnesoid X receptor (*Nr1h4*) develop spontaneous liver tumors (27, 75), and deficiency of *Nr0b2* (SHP) causes increased growth of hepatocellular carcinomas (25). Taken together, these findings show that the tissue specificity of transcriptional defects in *Hnf1a*-deficient islets and liver leads to opposed effects on cell growth and oncogenesis.

Identification of targets reveals general properties of direct *Hnf1 α* function. Gene expression studies cannot distinguish direct from indirect *Hnf1 α* -dependent effects in pancreatic islets and liver. At the same time, genomic binding studies cannot distinguish functionally essential and nonessential binding events. To understand how *Hnf1 α* controls cellular programs *in vivo*, we studied the functional consequences of its direct interactions with genes.

Our analysis of *Hnf1 α* binding by ChIP was restricted to 5' flanking regions. In all likelihood, *Hnf1 α* also regulates several genes through binding to more-distant sites. However, in practice, a distantly located binding site often cannot be unambiguously linked to its gene target, whereas binding to proximal promoter regions provides a reasonable degree of certainty as to the regulatory target. Our experiments thus obtained a collection of genes that are highly enriched in bona fide direct *Hnf1 α* targets.

In a complementary approach, we performed a computational genome scan that revealed a remarkable enrichment of conserved HNF1 motifs in the proximal promoters of genes that were downregulated in *Hnf1a*^{-/-} hepatocytes and islets. Although a recent report showed that *Hnf1 α* binding diverges in many mouse and human orthologs (43), we observed that binding conservation is very high among genes with perturbed expression in *Hnf1a*-deficient mouse or human samples (5). Our results thus enabled us to use conserved 5' flanking HNF1 motifs in regulated genes as a proxy for direct *Hnf1 α* targets.

The combination of expression, computational, and ChIP analyses allowed us to address basic questions concerning how *Hnf1 α* regulates its direct targets. First, we found that *Hnf1 α* is essential for gene expression and histone 3-K4 methylation in only a minor fraction of its direct targets. Recent studies have also shown lack of changes of gene expression for a large fraction of genes that are bound by transcription factors when they are perturbed in loss-of-function models (33, 74). This is likely to reflect redundant roles of other DNA-binding activators acting on the same genes, although some binding events could be truly nonfunctional, as recently proposed for several *Drosophila* regulators (32). Such findings highlight that the study of transcription factor occupancy alone is insufficient for understanding gene regulatory programs.

We also established a major role for *Hnf1 α* as a transcrip-

tional activator. Previous studies showed that Hnf1 α is a transcriptional activator (8). However, transcription factors often exert dual roles, and some reports suggest that Hnf1 α may have direct repressor functions (53, 61). Because we observed no enrichment of Hnf1 α binding among genes that were up-regulated in *Hnf1a*-deficient tissues, we believe that Hnf1 α does not play an essential repressor role at its directly bound targets. An indirect interference of Hnf1 α with the activity of other activators nevertheless remains possible.

The integration of expression and binding experiments has therefore provided novel insights concerning how Hnf1 α exerts its transcriptional functions in vivo. Importantly, direct transcriptional functions were mildly but significantly impaired in haploinsufficient islets. Although the outcome of this perturbation is insufficient to cause diabetes in mice, it suggests that the conclusions derived from *Hnf1a* homozygous null-mutant islets are relevant to the *HNFLA*-deficient, haploinsufficient, diabetic phenotype.

Hnf1 α controls tissue-specific genetic programs. The integrated data sets also revealed different mechanisms whereby a single regulator controls different genetic programs in pancreatic islets and liver. Many tissue-specific expression changes in *Hnf1a*^{-/-} mice were likely indirect responses. For example, the islet-specific defect in glucose metabolism is expected to have major consequences on glucose-dependent gene expression (15, 45, 57). In keeping with this notion, glucose induction of gene expression is profoundly impaired in *Hnf1a*^{-/-} islets (unpublished results). This is most likely due to abnormal glucose metabolism rather than to a direct function of Hnf1 α , because although selected Hnf1 α -bound genes are regulated by glucose, Hnf1 α binding was not enriched among the overall set of glucose-dependent genes (unpublished results).

Tissue-specific regulation is also expected because Hnf1 α selectively binds to genes that are expressed only in either islets or liver. Another mechanism, however, can be linked to the findings that Hnf1 α is an essential activator in only a subset of its bound genes and that the subset of targets that requires Hnf1 α is highly tissue specific. Intriguingly, we found that genes that are expressed in both pancreatic islets and liver often require Hnf1 α only in pancreatic islets.

Our findings indicate that Hnf1 β can partly explain such tissue-specific requirements for Hnf1 α . Hnf1 α and Hnf1 β are paralogs with known interchangeable functions in several contexts (24, 29). In *Hnf1a*-deficient liver, Hnf1 β was induced and occupied a subset of direct Hnf1 α target genes that maintained normal expression despite the absence of Hnf1 α . However, these same genes were downregulated in *Hnf1a*^{-/-} islets, where Hnf1 β is not expressed. In contrast, Hnf1 β binding was not induced in liver-selective genes that are downregulated in *Hnf1a*^{-/-} mice. Future studies are needed to address why certain liver-specific genes appear to be selectively dependent on the specific transactivator functions of Hnf1 α , which are distinct from those of Hnf1 β (8).

In summary, the final phenotypic outcome of *Hnf1a* deficiency is highly cell specific and results from an integrated failure of multiple direct and indirect functions of Hnf1 α in pancreatic islets and liver. The breadth of *Hnf1a*-dependent transcriptional programs suggests that to correct the defects causing β -cell dysfunction in *HNFLA*-deficient diabetes, it will be necessary to manipulate proteins or pathways acting on the

β -cell *Hnf1a*-dependent program rather than to restore the activity of individual target gene products.

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