

Developmental Gene Expression in the Human Fetal Pancreas

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ABSTRACT

Differential developmental regulation of pancreas-specific genes has not been reported for the human fetal pancreas. We have therefore undertaken a systematic, quantitative analysis of the transcriptional levels of various genes in the human pancreas at different stages of fetal and postnatal development. Using sensitive ribonuclease protection assays, *in situ* hybridization, and the polymerase chain reaction, our results indicate the following: 1) Transcriptional levels of insulin and amylin remain lower in the fetal than in the adult pancreas, whereas glucagon and somatostatin mRNA levels are consistently greater after 14 wk gestation than postnatally. These results are in agreement with previous immunohistochemical studies of these gene products. 2) The *reg* gene exhibits a 20-fold increase in mRNA levels after 16 wk gestation. The gene is expressed exclusively in the acinar cells and does not colocalize with insulin. This restricted exocrine expression does not indicate a direct role for the *reg* gene in islet

development. 3) Glucose transporter 2 and glucokinase mRNA are detectable as early as 13 wk gestation and remain low throughout development. Glucose transporter 1 reaches adult transcriptional levels by 18 wk gestation. The early detection of glucose transporter 2 and glucokinase implies that lack of expression of these "glucose sensor" genes does not account for the known insensitivity of the fetal β -cells to glucose. (*Pediatr Res* 36: 537-544, 1994)

Abbreviations

reg gene, regenerating gene
GLUT-1 or -2, glucose transporter 1 or 2
RT-PCR, reverse-transcription polymerase chain reaction
RNase, ribonuclease
UTR, untranslated region
nt, nucleotide
tRNA, transfer RNA

The developing human fetal pancreas has been the subject of many immunohistochemical and ultrastructural studies. The appearance and frequency of the different endocrine cell types vary during development, and it is now generally accepted that the α -cells appear first at approximately 8-9 wk gestation, followed sequentially by the β -cells, δ -cells, and PP-cells. By 9-10 wk gestation, all four pancreatic endocrine cell populations have been detected by immunofluorescence, immunocytochemistry, and electron microscopy (1-4).

Studies in other systems, including the mouse pancreas, have shown that temporal patterns of gene expression can differ significantly from those detected by techniques that determine the presence of protein. Using a combination of embryo microdissection and the polymerase chain reaction, Gittes and Rutter (5) observed that

transcription of the endocrine genes occurred before detectable morphogenesis in the mouse. It was suggested that this "premorphogenetic phase" of organogenesis may represent the beginning of the endocrine cell lineages. A similar situation may also occur in the human pancreas.

Insulin release from the pancreatic islets is controlled by the uptake and metabolism of glucose by the β -cells. Although insulin is present in the human fetal pancreas from 10 wk gestation, insulin release remains practically unresponsive to glucose stimulation until birth (6). The cellular uptake of glucose across the plasma membrane is accomplished by membrane-associated carrier proteins, termed glucose transporters. GLUT-1 is the predominant isoform expressed in most fetal tissues, whereas GLUT-2 is the major isoform detected in adult pancreatic β -cells (7-9). It has been suggested that the glucose-sensing apparatus of β -cells consists of GLUT-2 working in concert with glucokinase, the enzyme that catalyzes the rate-limiting step in glucose metabolism (8, 10). Expression of the genes encoding GLUT-1, GLUT-2, and glucokinase has not been reported during human fetal pan-

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creatic development; therefore, it was important to examine their expression levels in light of the reported glucose insensitivity of the fetal β -cell.

Since the identification of the reg gene in adult rat islets undergoing regeneration (11), its physiologic role has remained unclear, with evidence for both exocrine and endocrine involvement (12–15). Studies on the expression of the reg gene in the human fetal pancreas may help clarify its role in pancreatic development.

This study was undertaken to obtain a more complete understanding of pancreatic ontogeny as it relates to the transcriptional regulation of pancreatic genes. We present detailed quantitative data on the developmental regulation of the steady state levels of the mRNA encoding the islet hormones, as well as other pancreas- or islet-specific proteins in the human fetal and postnatal pancreas.

METHODS

Tissue procurement. Intact human fetal pancreata at 12–24 wk gestational age were obtained through nonprofit organ procurement centers (Advanced Bioscience Resources, Oakland, CA; International Institute for the Advancement of Medicine, Exton, PA). Informed consent for tissue donation was obtained by the procurement centers. Gestational age was determined by several criteria, including biparietal diameter, femur length, and fetal foot measurements. Pancreatic tissues arrived in our laboratory after periods of 5 min and 24 h of warm and cold ischemia, respectively. Tissues were shipped on ice in RPMI 1640 medium (Irvine Scientific, Irvine, CA) containing 10% pooled normal human serum and antibiotics (100 U/mL penicillin, 0.1 mg/mL streptomycin, and 1 μ g/mL amphotericin B). Upon arrival, pancreatic tissues were carefully dissected free of extraneous tissue, immediately snap-frozen in liquid nitrogen, and stored at -70°C . Adult pancreatic tissue was kindly provided by Dr. J. Chang (Immune Response Corp., Carlsbad, CA). Adult pancreas and liver RNA were obtained from Clontech Laboratories (Palo Alto, CA). A pancreatic section obtained at autopsy (4 h postmortem) of an 11-mo-old child who died of a brain tumor was provided by Children's Hospital of San Diego. Adult islets were kindly provided by Dr. C. Ricordi (University of Pittsburgh Transplant Institute, Pittsburgh, PA).

RNAse protection assay probes. All probes used for the RNAse protection assay were of human origin and were subcloned into the pGEM (Promega, Madison, WI) or pBluescript (Stratagene, La Jolla, CA) riboprobe vectors by standard procedures (16). The insulin probe (provided by Dr. P. Miettinen, University of Helsinki, Finland) was a 262-bp fragment corresponding to amino acids 1–87 in preproinsulin mRNA (17). The glucagon probe (provided by Dr. D. Drucker, Toronto General Hospital, Toronto, Ontario, Canada) encompassed 5'UTR sequences and amino acids 1–117 in preproglucagon mRNA (18). Somatostatin (ATCC, Rockville, MD) was detected by a

subclone representing bp 1245–1480 in the genomic sequence (19). The cyclophilin probe (provided by Dr. D. Bergsma, SmithKline Beecham Pharmaceuticals, King of Prussia, PA) corresponded to 5'UTR sequences and amino acids 1–28 in the full-length cDNA sequence (20). Amylin was detected by a subclone representing a portion of the 5'UTR and amino acids 1–66 in the cDNA sequence (provided by Dr. D. Steiner, Howard Hughes Medical Institute, University of Chicago, Chicago, IL). The amylase probe (ATCC) was a genomic subclone composed of a portion of intron 1, the entire exon 1, and part of intron 2 (21). The reg gene probe (provided by Dr. H. Okamoto, Tohoku University School of Medicine, Sendai, Japan) corresponded to amino acids 115–165 and 3'UTR sequences (11). The GLUT-1 probe (ATCC) consisted of 5'UTR sequences and amino acids 1–93 in the cDNA sequence (22). Plasmid constructs were linearized with the appropriate restriction enzymes and gel purified using the QIAEX gel extraction kit (QIAGEN Inc., Chatsworth, CA). Radiolabeled antisense probes were generated by *in vitro* transcription in the presence of [^{32}P]uridine triphosphate using the appropriate RNA polymerase (23). Protected probe lengths are as follows: insulin, 262 nt; glucagon, 389 nt; somatostatin, 236 nt; cyclophilin, 135 nt; amylin, 267 nt; amylase, 214 nt; reg, 180 nt; and GLUT-1, 294 nt.

RNA isolation and analysis. Total cytoplasmic RNA was isolated by the acid guanidinium thiocyanate method (24) and quantitated spectrophotometrically. Transcriptional analyses were performed using a multiprobe RNase protection assay (23, 25). Total RNA (0.4–5 μ g) was hybridized for 18 h at 56°C with ^{32}P -labeled antisense RNA probes in 40 mmol/L PIPES buffer (pH 6.4) containing 400 mmol/L NaCl, 1 mmol/L EDTA, and 80% formamide. Yeast tRNA (10 μ g) was included as a negative control. Excess probe and nonhomologous RNA sequences were removed by RNase digestion (50 μ g/mL RNase A and 50 U/mL RNase T1) in 10 mmol/L Tris-HCl (pH 7.5), 300 mmol/L NaCl, and 5 mmol/L EDTA for 1 h at 30°C , followed by treatment with 200 μ g/mL proteinase K in 2% SDS for 30 min at 37°C . Samples were extracted with phenol/chloroform, ethanol precipitated with 10 μ g of yeast tRNA as carrier, and dissolved in formamide sample buffer. The protected, double-stranded hybrids were separated by denaturing PAGE, followed by autoradiography on Kodak XRP film to visualize the target RNA. Quantitation was performed by scanning densitometry (LKB UltraScan XL Laser) and integrated using GelScan XL software (Pharmacia LKB, Piscataway, NJ). Peak heights were used to quantitate transcriptional levels. Different exposure times were used to ensure that all signals fell within the linear range of the densitometer. The probe-specific mRNA signals were normalized to the cyclophilin signal in each sample to account for differences in sample loading between lanes.

Tissue preparation for *in situ* hybridization and immunohistochemistry. A 24-wk gestational age fetal pancreas was fixed in 4% paraformaldehyde in PBS (pH 7.4) at 4°C for

4 h. After dehydration, the tissue was embedded in paraffin and 5- μ m sections were mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). Consecutive sections were used for both *in situ* hybridization and immunohistochemistry.

In situ hybridization. Sense and antisense probes for both insulin and the reg gene were transcribed from cDNA in the presence of [35 S]uridine triphosphate using the appropriate RNA polymerase. *In situ* hybridization was performed according to the method of Simmons *et al.* (26). Tissue sections were digested with proteinase K (10 μ g/mL) for 30 min at 37°C, acetylated for 10 min at room temperature, rinsed in 0.3 mol/L NaCl–30 mmol/L sodium citrate (pH 7.0), dehydrated, and air dried. Hybridization with the 35 S-labeled probes ($2\text{--}4 \times 10^6$ cpm/mL) was performed at 55°C for 16 h in a buffer consisting of 50% formamide, 0.3 mol/L NaCl, 10 mmol/L Tris (pH 8.0), 1 mmol/L EDTA, 1 \times Denhardt's solution (0.02% each of Ficoll 400, polyvinylpyrrolidone, and BSA, Fraction V), 10% dextran sulfate, 50 mmol/L DTT, and 500 μ g/mL each of yeast tRNA and *Torula* yeast RNA. Sections were digested with RNase A at 37°C for 30 min, washed under high-stringency conditions, dehydrated in ethanol, and dried. Slides were coated with Kodak NTB-2 autoradiograph emulsion and exposed in sealed boxes at 4°C for 10 d. After development in Kodak D-19 for 3.5 min, the slides were rinsed, fixed, and counterstained with hematoxylin and eosin. Hybridization signals were analyzed using bright- and dark-field microscopy.

Immunohistochemistry. Paraffin sections (5 μ m) from the 24-wk gestational age fetal pancreas were stained for insulin using the immunoalkaline phosphatase technique (27). Briefly, deparaffinized and rehydrated sections were

blocked with 10% goat serum for 10 min, washed, and incubated with polyclonal guinea pig anti-porcine insulin antibody (Chemicon, El Segundo, CA) at a 1:100 dilution for 3 h. After washing, sections were incubated for 20 min with a biotinylated goat anti-rabbit antibody that cross-reacts with guinea pig antibody (BioGenex, San Ramon, CA), washed, and incubated with streptavidin alkaline phosphatase for 20 min. Immunoreactivity was visualized using Fast Red (Sigma Chemical Co., St. Louis, MO) as chromogen, then sections were washed and counterstained with hematoxylin.

RT-PCR. Total RNA (1–2 μ g) from fetal pancreatic tissues (13 wk gestation), adult islets, and adult liver was reverse transcribed into cDNA with an oligo d(T) primer according to manufacturer's recommendations (GeneAmp RNA PCR kit, Perkin-Elmer Cetus, Norwalk, CT). The samples were divided in two and amplified with GLUT-2 and glucokinase primers. Primers specific for human GLUT-2 and islet-specific glucokinase were as previously described (28–30) and were designed to span intron sequences to distinguish amplification products resulting from any contaminating genomic DNA sequences present in the RNA preparations. Amplifications were performed in a PowerBlock temperature cycler (Ericomp, San Diego, CA). Samples were initially denatured at 94°C for 1 min. Cycling parameters (45 cycles) were as follows: denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min (GLUT-2), or annealing and extension simultaneously at 72°C for 1 min for glucokinase. A final extension at 72°C for 7 min was performed. Portions (10%) of the amplified products were analyzed by electrophoresis, in the presence of ethidium bromide, on 3% NuSieve GTG/1% agarose gels (FMC, Rockland, ME).

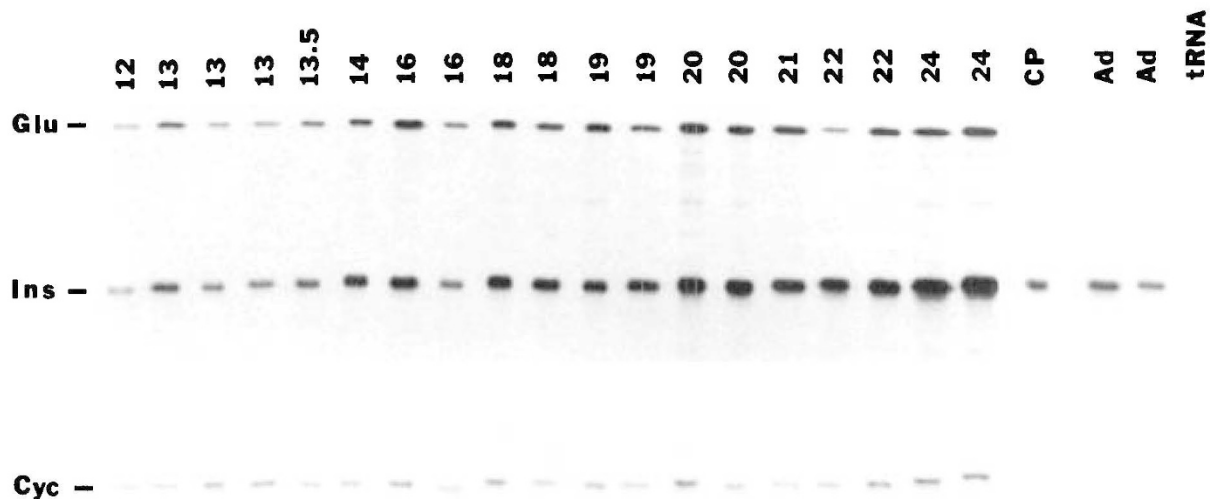


Figure 1. RNase protection analysis of hormone transcripts in a series of fetal and postnatal pancreata. Total RNA (400 ng) was hybridized simultaneously to antisense riboprobes for glucagon (*Glu*), insulin (*Ins*), and cyclophilin (*Cyc*) for 18 h at 56°C as described in Methods. Lanes for fetal tissue samples are identified by their gestational ages, in wk (12–24); *CP*, RNA from an 11-mo-old child; *Ad*, adult pancreas; tRNA, yeast tRNA (used as a negative control). Protected fragments for glucagon (389 nt), insulin (262 nt), and cyclophilin (135 nt) are indicated. Exposure times were 4 h for glucagon and insulin and 16 h for cyclophilin. Extended exposure times were required to detect glucagon transcripts in the 11-mo-old and adult pancreas samples.

RESULTS

Pancreatic hormone expression. Transcriptional expression of the genes encoding insulin or glucagon (Fig. 1) and somatostatin (Fig. 2A) were examined in a series of human fetal pancreata, ranging in gestational ages from 12–24 wk, using a multiprobe RNase protection assay. Quantitation of the hormone-specific mRNA was performed by scanning densitometry, normalizing the probe-specific hybridized signal to the signal from the ubiquitous housekeeping gene cyclophilin (Table 1). The absence of any hybridized signals in the negative control (yeast tRNA) indicates the specificity of the assay. Insulin transcriptional levels continued to increase through gestation, with 3- to 5-fold more insulin mRNA present at 24 wk compared with 12 wk, yet remained lower than levels found in the adult pancreas (Table 1). In contrast, glucagon transcriptional levels peaked at approximately 20 wk gestational age and remained approximately 3-fold greater than adult levels. Somatostatin mRNA levels were consistently greater after 14 wk gestation than adult levels, which were barely detectable using equivalent RNA amounts (Table 1).

Amylin and amylase gene expression. Because amylin is cosecreted with insulin in the adult pancreas in response to glucose, it was of interest to determine its expression pattern throughout fetal development. The levels of amylin transcripts were fairly constant, as opposed to the gradual increase in insulin transcripts, throughout the gestational ages examined. An amylin transcript was initially seen at 13 wk gestational age and did not vary considerably throughout development (Fig. 2B). Amylin mRNA levels at 24 wk gestational age were approximately 10-fold lower than adult levels (Table 1). The pancreas from the 11-mo-old child had approximately 2-fold more amylin transcripts than the adult pancreas. No hybridization signal was seen in the negative control.

Amylase transcripts were not detectable in any of the fetal pancreata analyzed (data not shown). The adult pancreas had approximately 4- to 5-fold more amylase transcripts than the 11-mo-old pancreas (Table 1).

Expression of the reg gene. Transcriptional expression of the islet reg gene showed low expression levels before 16 wk gestation. At this time, however, a sudden increase in reg mRNA levels was seen and continued to increase through 24 wk gestational age, at which point it reached levels comparable to those in the adult pancreas (Fig. 2C, Table 1), which were 2-fold greater than those in the 11-mo-old pancreas.

Consecutive serial sections (5 μ m thick) from a 24-wk gestational age pancreas were hybridized to the insulin and reg gene riboprobes to identify their locations within the fetal pancreas. *In situ* hybridization with the insulin probe was used to identify the β -cells. Silver grains were specifically associated with scattered clusters of epithelial cells of variable sizes throughout the acinar tissue (Fig. 3A). Binding of the insulin sense probe showed very low background signals (Fig. 3B). The disseminated pat-

tern of β -cells throughout the epithelial components of the fetal pancreas was also evident by immunostaining for insulin (Fig. 3C). The reg gene signal was entirely localized within the acinar cells of the fetal pancreas (Fig. 3D-F). The clustering of specific hybridization signals resembled the pattern seen with the insulin probe. However, the reg gene signal was clearly more abundant than the insulin signal. Mesenchymal tissue and ductal epithelium were devoid of the reg gene signal. Consecutive sections hybridized with the control sense probe showed only low levels of nonspecific hybridization signals (not shown). To determine whether the insulin and reg genes are both expressed in the β -cells, consecutive serial tissue sections were hybridized to both probes. We found no evidence for colocalization of the insulin (Fig. 4A-C) and reg genes (Fig. 4D-F).

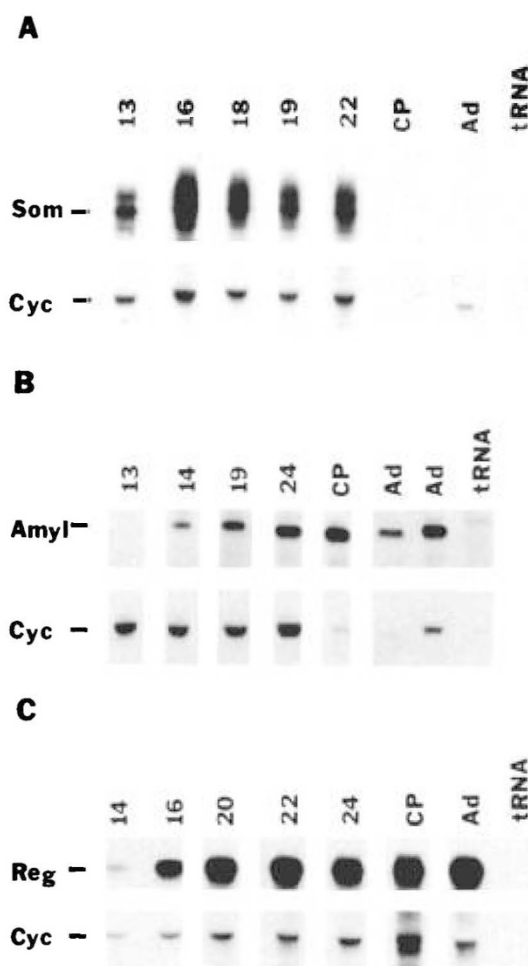


Figure 2. Detection of somatostatin, amylin, and reg gene transcripts in selected fetal and postnatal pancreata, demonstrating transcriptional modulation. RNase protection assays were performed on 400 ng of total RNA for somatostatin (*Som*; A), 2 μ g for amylin (*Amyl*; B), and 400 ng for reg gene (*Reg*; C) as described above. Identification of lanes is the same as in Figure 1. Sizes of protected fragments are 236 nt (somatostatin), 267 nt (amylin), 180 nt (reg gene), and 135 nt (cyclophilin). Exposure times were as follows: A, 54 h for somatostatin and 3 d for cyclophilin; B, 4 d for amylin and 18 h for cyclophilin; and C, 18 h for reg gene and cyclophilin. Longer exposure times were necessary to detect somatostatin transcripts in the 11-mo-old and adult pancreata.

Table 1. Quantitation of appearance and modulation of various genes during development of human fetal pancreas*

Gestational age	n	Insulin	Glucagon	Somatostatin	Amylin	Amylase	Reg	GLUT-1
12 wk	1	0.38	0.25	0.12	ND	ND	ND	
13 wk	3	0.34	0.32	0.23	0.02	ND	0.03	0.65
14 wk	2	0.58	0.49	0.28	0.41	ND	0.03	0.99
16 wk	2	0.63	0.83	1.00	0.09	ND	0.58	0.82
18 wk	2	0.88	0.78	1.64	0.08	ND	0.26	1.51
19 wk	2	0.84	0.94	1.11	0.47	ND	1.63	1.18
20 wk	3	1.40	1.44	1.56	0.26	ND	0.85	1.68
22 wk	2	1.35	0.59	1.74	0.80	ND	2.07	1.64
24 wk	2	1.59	0.86	1.79	0.59	ND	1.46	1.73
11 mo	1	2.50	0.17	0.10	11.25	1.78	0.75	0.70
Adult	2	2.40	0.40	0.43	5.67	8.23	1.56	1.35

* Values are the averages of the densitometric scans of autoradiographs from RNase protection assays at each age of the indicated number (*n*) of pancreata after normalization to the cyclophilin signal. Exposure times of the autoradiographs were varied to ensure that the signals were within the linear range of the densitometer. Each autoradiograph for all samples for a particular gene was scanned at the same exposure time. *ND*, not detected.

Glucose transporters and glucokinase expression. Expression of GLUT-1 transcripts in fetal pancreatic tissue was fairly constant through 16 wk gestational age, at which time an approximate 2-fold increase was measured (Fig. 5; Table 1). The level of GLUT-1 transcripts at 18–24 wk gestation was similar to adult levels.

GLUT-2 transcripts were not detectable in any of the fetal or adult pancreata using the RNase protection assay, indicative of their low abundance in these tissues. Very low expression levels of glucokinase mRNA were detected in all the pancreatic samples analyzed, with no quantitative differences between fetal and adult tissues (data not shown). We therefore used the more sensitive technique, RT-PCR, to identify when transcription of these genes was first detectable (Fig. 6). Both GLUT-2 and the islet-specific glucokinase transcripts were detected using RT-PCR in the 13-wk fetal pancreata (*n* = 3), the youngest tissue samples tested. Human adult islets and liver were used as positive controls for GLUT-2. The specificity of the primers for the islet-specific glucokinase was borne out by the presence of an amplified product in the adult islet sample and the absence of any amplified product in the liver sample.

DISCUSSION

This study represents the first comprehensive report on comparative gene expression in the human fetal and postnatal pancreas, illustrating the developmental appearance and modulation of pancreas-specific genes. We used sensitive and highly specific RNase protection assays, *in situ* hybridization, and the polymerase chain reaction to detect and quantitate transcripts for endocrine and exocrine genes in the pancreas.

Because insulin release in the human fetal pancreas is unresponsive to glucose stimulation (6), it was important to determine whether this was due to a lack of transcription of the β -cell-specific glucose-sensing apparatus, namely, GLUT-2 and glucokinase. It has been suggested that inappropriate expression levels of these genes may be responsible for the abnormal insulin secretion observed in diabetic rats (31, 32) and in cell lines (8, 33). In

normal β -cells, glucose transport capacity is in excess relative to glucose metabolism. In fact, it has been suggested that glucose transport into the β -cells needs to be reduced by at least 90% to interfere with glucose metabolism (34), supporting the notion that GLUT-2 plays only a permissive role in glucose sensing. Glucokinase has been proposed to be the β -cell “glucose sensor” (35, 36) because it catalyzes the rate-limiting step in glucose metabolism, which is a prerequisite for insulin secretion. Our RNase protection assay and RT-PCR data indicated the presence of GLUT-1, GLUT-2, and glucokinase transcripts in the 13-wk fetal pancreata, suggesting the presence of the respective functional proteins. Thus, it would appear that fetal β -cells are capable of transporting and phosphorylating glucose. However, we cannot rule out quantitative differences in these processes between the fetal and adult pancreas.

The role of the reg gene and its protein product is unclear in human fetal pancreatic development. The reg protein has been colocalized with insulin within the secretory granules of the regenerating β -cell (37). Based on these findings, and supported by later work with isolated rat islets (14), a role has been suggested for the reg gene in pancreatic islet growth and regeneration. However, other investigators have found the gene to be expressed in the exocrine portion of the adult pancreas (12, 15), where it encodes pancreatic stone protein, a major constituent of the exocrine pancreatic secretion (38). In the human fetal pancreas, the reg protein has previously been detected in the acinar cells, which were found to be weakly immunoreactive from 16 to 27 wk gestational age, at which point the staining became more intense, and markedly increased at birth (39). We observed very low expression of the reg gene before 16 wk gestation, at which time a dramatic increase was seen. The levels remained high throughout gestation and reached levels comparable to those in the adult pancreas. Results of our *in situ* hybridization analysis are in agreement with the results of Miyaura *et al.* (13); that is, reg genes are entirely localized within the acinar tissue and are absent from mesenchymal, ductal, and islet cells. Additionally,

reg transcripts did not colocalize with insulin in the β -cells, unlike the results of Terazono *et al.* (37). The barely detectable levels of reg mRNA in the 12- to 14-wk fetal pancreata, which already contain significant levels of the hormone transcripts, suggest that the reg gene does not play a direct role in early islet development. However, the dramatic increase in reg transcripts seen at 16 wk gestation, an age at which there is no significant expansion of the exocrine pancreatic compartment, may implicate the reg gene as being involved in the expansion of the islet mass. This is supported by Francis *et al.* (14), who showed an increase in reg mRNA expression associated with adult rat islet cell replication. Although the function of the reg gene product is unknown, prediction of its three-dimensional structure indicates significant homology with plant and animal lectins, suggesting a possible growth-promoting role (40, 41). Additional work

is needed to more clearly define the role of the reg gene in pancreatic development.

The levels of islet hormone transcripts (insulin, glucagon, and somatostatin) increased gradually throughout fetal development. Insulin mRNA levels in the fetal pancreas were found to be 2- to 6-fold lower compared with those in the postnatal pancreas, in contrast to glucagon and somatostatin mRNA levels, which were higher in the fetal than in the postnatal pancreas. These results are in agreement with previously published immunohistochemical and ultrastructural data (1, 3, 4). The physiologic impact of the relatively high glucagon and somatostatin expression in the fetus is not clear. Transcription levels of the three hormones were similar in the pancreas of the 11-mo-old child and the adult, indicating that islet hormone expression matures completely during the first year of life.

In the normal adult pancreas, amylin is colocalized with insulin in the secretory granules (42, 43), where it is released concomitantly with insulin in response to a glu-

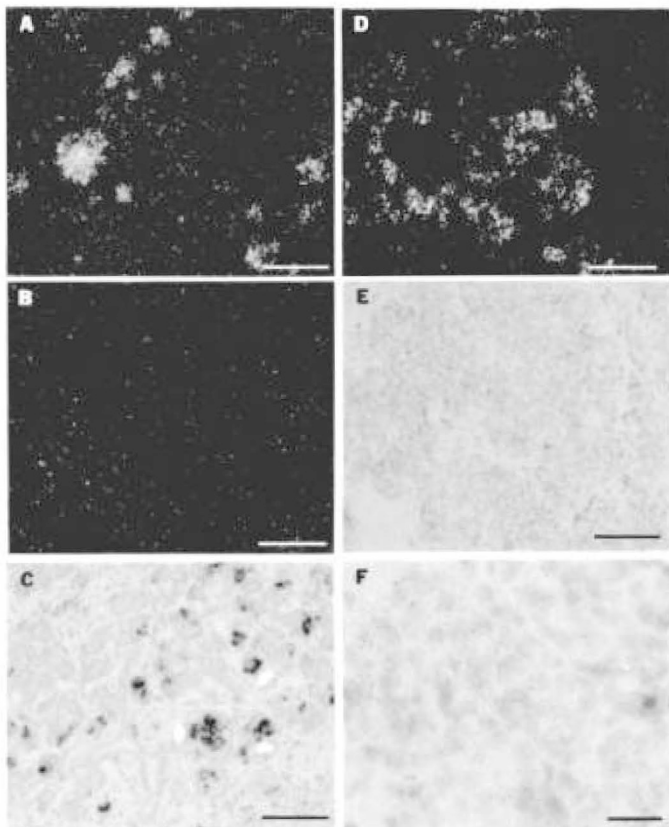


Figure 3. Localization of insulin and reg gene transcripts in a 24-wk gestational fetal pancreas using *in situ* hybridization. Dark-field micrographs of consecutive sections hybridized with the insulin antisense (A) or control sense (B) probes, identifying the β -cells in the fetal pancreas, are shown. Low magnification of the dark-field image (A) shows specific association of silver grains with those epithelial cells expressing insulin mRNA. Note the lack of specific hybridization signal when the control sense cRNA probe was used (B). Immunohistochemical staining for insulin (C) demonstrates that the β -cells appear as small clusters of variable sizes scattered throughout the epithelial tissue (arrows). Low magnifications of dark- (D) and bright-field (E) images of a fetal pancreatic tissue section hybridized with the reg gene probe are shown. Note the clustering of specific signals associated with epithelial cells. F, Higher magnification of panel E showing those acinar cells expressing reg gene mRNA. Bars: A-E, 100 μ m; F, 20 μ m.

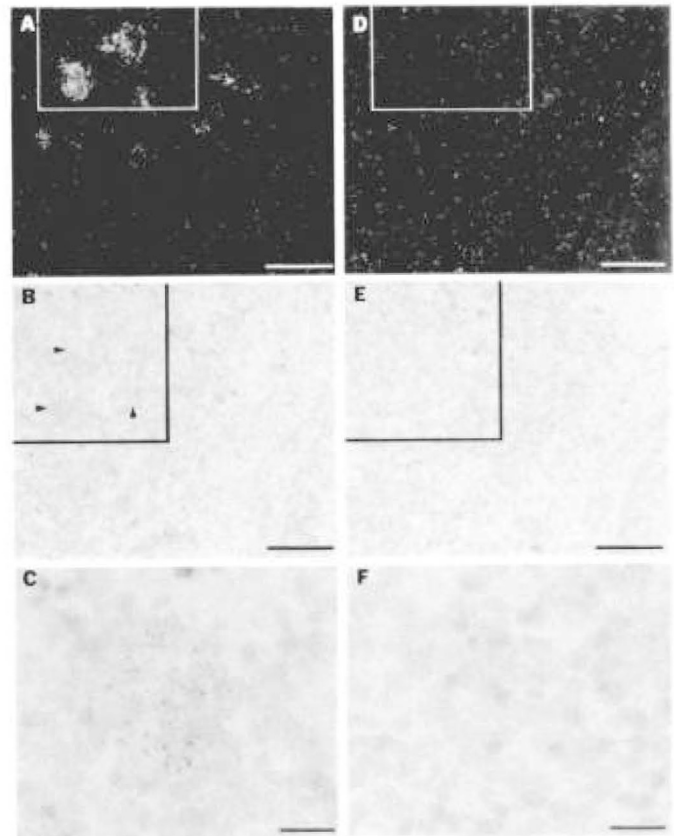


Figure 4. *In situ* hybridization analysis demonstrating that the insulin and reg genes do not colocalize in the fetal β -cells. Dark-field (A and D) micrographs of the same fields from consecutive tissue sections of a 24-wk gestational pancreas hybridized with the insulin (A) or reg gene (D) probes are shown. The boxed areas show the lack of reg gene expression in the insulin-expressing acinar cells. Panels B and E are the corresponding bright-field micrographs of panels A and D, respectively, showing clusters of silver grains over groups of acinar cells (arrowheads). Panel C is a higher magnification of panel B showing the specific association of the insulin probe with the acinar cells. Note the absence of reg gene expression (F) in the same group of acinar cells that express insulin (C). Bars: A, B, D, and E, 100 μ m; C and F, 20 μ m.

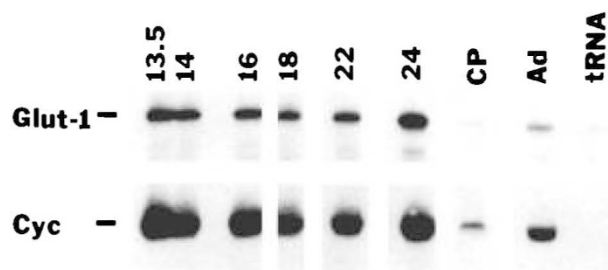


Figure 5. Detection of GLUT-1 transcripts in selected fetal and postnatal pancreata. Total RNA (5 μ g) was hybridized to the GLUT-1 riboprobe as described above. Identification of lanes is the same as in Figure 1. Representative fetal pancreas samples are shown, illustrating increased transcriptional levels in the latter gestational ages. Exposure times were 65 h for GLUT-1 and 17 h for cyclophilin (*Cyc*).

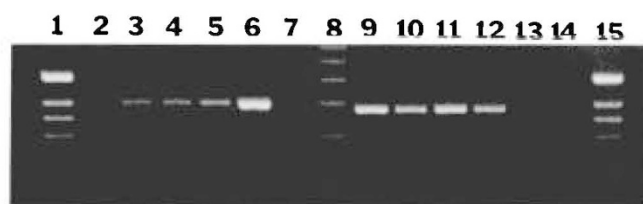


Figure 6. Detection of GLUT-2 and glucokinase transcripts using RT-PCR in 13-wk gestational age pancreata. Total RNA (1.3 μ g) from three different 13-wk pancreata was reverse transcribed into cDNA; aliquots were then amplified as described in Methods, analyzed on agarose gels, and visualized by ethidium bromide staining. Molecular mass markers are a 1-kb DNA ladder (BRL, Grand Island, NY), lanes 1 and 15, and a 100-bp ladder (GenSura Laboratories, San Diego, CA), lane 8. GLUT-2 (lanes 2-4; 398 bp) and glucokinase (lanes 9-11; 380 bp) transcripts were detected using RT-PCR in three different 13-wk fetal pancreata. Adult islets were used as positive controls for GLUT-2 (lane 5) and glucokinase (lane 12). Adult liver was used as a positive control for GLUT-2 (lane 6) and as a negative control for glucokinase (lane 13), illustrating the specificity of the polymerase chain reaction primers for the islet-specific glucokinase. Water was used as a negative control (lanes 7 and 14).

cose stimulus. We first detected amylin mRNA at 13 wk gestation, in agreement with initial amylin immunoreactivity at 13 wk (44), considerably later than insulin immunoreactivity, which occurred at 9 wk gestation. In the previously published immunohistochemical study, the percentage of amylin-positive insulin cells increased with gestational age and reached nearly 100% in the adult pancreas (44). In accordance with this, we observed an approximately 10-fold increase in amylin transcripts in the adult compared with the fetal pancreas.

The amniotic fluid swallowed by the fetus acts as a stimulus for the developing exocrine pancreas. The non-parallel appearance of secretory proteins in the fetal exocrine pancreas has previously been reported (39, 45, 46), perhaps reflecting the adaptive ability of the pancreas to the changing composition of the amniotic fluid. Inasmuch as amniotic fluid is devoid of starch, it is not surprising that amylase activity has not been detected in the fetal pancreas (45, 46). In fact, amylase was not detectable in the neonatal pancreas either (46, 47), but did increase during the first year (48). Consistent with this

developmental process, we were unable to detect expression of the amylase gene in the fetal pancreas, although high levels of amylase transcripts were detected in the pancreas of the 11-mo-old child and the adult.

In summary, we measured transcriptional expression of various endocrine and exocrine genes in the human pancreas during development. Our results confirmed earlier immunohistochemical and enzymatic studies of the islet hormones, amylin, and amylase. In addition, we showed that the *reg* gene is developmentally regulated in the human fetal pancreas, with an abrupt increase in expression at 16 wk gestation, and that the gene localizes exclusively to the pancreatic acinar cells. Furthermore, we showed that the GLUT-1, GLUT-2, and glucokinase genes are expressed early in human pancreatic development. This study establishes a baseline of information on the temporal transcriptional regulation of pancreas-specific genes during pancreatic ontogeny and contributes to the basic research and knowledge required before undertaking human fetal tissue transplantation as a means to treat insulin-dependent diabetes.

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