Insulin Gene Expression during Development of the Fetal Bovine Pancreas†

Marsha L. Frazier, Richard A. Montagna, and Grady F. Saunders*

ABSTRACT: Poly(A+) RNA was isolated from the bovine pancreas at three stages of fetal development. Approximately 1% of the total RNA from first, second, and third trimester fetuses was polyadenylated, and the mean chain length of each RNA population was 1350 nucleotides. In cell-free protein synthesis experiments the concentration of insulin-immunoreactive translation products was 10.2%, 11.3%, and 9.7% for first, second, and third trimesters, respectively. Insulin mRNA sequences were estimated by transcription of insulin mRNA to [^{3}H]cDNA and hybridization of cDNA with plasmid pI19 DNA containing rat proinsulin I sequences. Hybridization experiments gave insulin mRNA concentrations of 7.6%, 12.9%, and 3.9% for first, second, and third trimesters, respectively. These results show that insulin mRNA levels vary during development and become proportionally lower in third trimester, when the exocrine tissue is rapidly increasing in mass.

Insulin-dependent diabetes mellitus involves a loss in pancreatic β cell function. The process of β cell development and the regulation of insulin synthesis in the pathogenesis of this disease are not well understood. A general morphological and biochemical view of both exocrine and endocrine pancreas development in rodents has been developed. The pancreas originates as an invagination of the primitive gut during the first trimester of gestation. This structure, the pancreatic diverticulum, composed of a single layer of endoderm surrounded by mesodermal cells, constricts and the epithelial cells proliferate away from the gut, resulting in the primitive organ called the pancreatic rudiment.

Tissue-specific gene products accumulate in a biphasic pattern during embryonic development of the rat pancreas (Rutter et al., 1968; Sanders & Rutter, 1974). The enzymatic activities characteristic of adult pancreatic secretion are present at relatively low, constant levels in the pancreatic rudiment between days 12 and 14 of gestation. Between 15 days of gestation and term (22 days) the specific activities of the pancreatic exocrine proteins increase several thousandfold as a consequence of at least a 100-fold increase in their rates of synthesis. It is assumed that this synthetic program arises from modulation of the rate of transcription of specific genes.

The availability of appropriate molecular probes allows investigation of the regulation of pancreatic β cell specific products and various aspects of normal and abnormal development associated with diabetes. In order to examine the expression of the bovine insulin gene during fetal development we have isolated poly(A+) RNA from bovine pancreata at various stages of development. Approximations of the relative insulin mRNA concentration at each of these stages of development are made through the utilization of molecular hybridization probes and analysis of in vitro translation products of poly(A+) mRNA. These measurements of relative insulin mRNA concentrations provide the necessary foundation for studies on transcriptional regulation of the bovine insulin gene.

Methods and Materials

Isolation of RNA. Fetal bovine pancreata were obtained at a local slaughterhouse from cows dead less than 30 min. Before removal of the pancreas, each fetus was weighed and measured in order to estimate its stage of development. All tissue was rapidly frozen in dry ice and maintained at −70 °C until use. Total nucleic acids were extracted by a modification of a method previously described (Lomedico & Saunders, 1976). Three to four grams of frozen tissue were homogenized for 5 min in a Waring blender with 88 mL of extraction buffer (75 mM NaCl, 25 mM EDTA, 0.1% $NaDodSO_4$, and 10 mM Tris-HCl, pH 8.0) and 112 mL of buffer-saturated phenol. The sample was then centrifuged 5 min at 5000*g* and the aqueous phase was treated with proteinase K, 100 μg/mL for 45 min at 37 °C. This was followed by a 10-min extraction with an equal volume of buffer-saturated phenol–Sevag (phenol–chloroform–isoamyl alcohol, 50:48:2). The aqueous phase was recovered by centrifugation and brought to 0.2 M NaCl, and two and one-half volumes of 95% ethanol were added. The sample was placed at −20 °C for 12 h. The precipitated nucleic acids were collected by centrifugation and dissolved in water and 2 volumes of 4.5 M sodium acetate, pH 6.0, were added. The RNA was precipitated by a 4-h incubation at −20 °C and collected by centrifugation. This treatment was repeated two additional times. After a final ethanol precipitation, the RNA was dissolved in sterile H_2O and stored at −20 °C until needed.

Preparation of Poly(A+) RNA. Poly(A+) RNA was isolated from total RNA by affinity chromatography of oligo(dT)–cellulose as previously described (Lomedico & Saunders, 1976).

Agarose Gel Electrophoresis. RNA was dissolved in 6.0 M urea–0.01% bromophenol blue and loaded onto cylindrical 2% agarose gels in 6.0 M urea–0.025 M citric acid, pH 3.5. Electrophoresis was carried out for 6 h at 2 mA/gel. The upper and lower buffer reservoirs were filled with 0.025 M citric acid, pH 3.5. The gels were stained either with methylene blue (0.02%) or ethidium bromide (0.5 μg/mL), depending on the amount of RNA analyzed.

Cell-Free Translation and Analysis of Translation Products. Translation of poly(A+) RNA in the wheat germ cell-free translation system was performed according to the procedure of Davies et al. (1977). High specific activity [^{3}H]leucine (65 Ci/mmol) was used at a concentration of 3.4 μM. Eleven microcuries of [^{3}H]leucine was added in each 50 μL assay. Immunoreactive insulin (IRI) was detected by immunoprecipitation of translation products by using the double-antibody technique described previously (Lomedico et al., 1977).

When rabbit reticulocyte lysate commercially prepared by New England Nuclear Corp. was used, the translation was

† From the Department of Biochemistry, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030. *Received April 25, 1980; revised manuscript received September 2, 1980.* This research was supported by grants from the KROC Foundation, U.S. Public Health Service (AM 20383, CA 16672), Robert A. Welch Foundation, and the Redfern Fund. M.L.F. is a National Institutes of Health postdoctoral fellow (F32-AM 6316).

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carried out as described in the NEN product description. Before gel electrophoresis, the translation products were adjusted to 1% $NaDodSO_4$, 5% β-mercaptoethanol, and 65 mM Tris-HCl, pH 6.8 and dialyzed overnight by a Pope microdialyzer against 0.1% $NaDodSO_4$, 0.1% β-mercaptoethanol, 0.065 M Tris-HCl, pH 6.8, and 15% glycerol. The sample was heated for 2 min at 100 °C and applied to a 10% polyacrylamide Laemmli slab gel prepared and run as described by Montagna & Becker (1978). After overnight electrophoresis at 80 V, the gel was treated for fluorography by sequentially suspending the gel for 1 h each in 50% methanol–10% acetic acid, NEN Enhance solution, and cold H_2O. The gel was then dried for 2 h in a slab gel drier and placed on a preflashed Kodak X-Omat film for 36 h.

Estimation of Poly(A) Content. The content of poly(A) present in the isolated poly(A+) RNA was determined by the method of Monahan et al. (1976). Briefly, a standard curve was generated by hybridizing known amounts of poly(rA) with 50000 cpm of [^{3}H]poly(dT) (10^8 cpm/μg). Hybrid formation was quantitated following S_1 digestion. Poly(A+) RNA was also hybridized under indentical conditions with excess [^{3}H]poly(dT) and the poly(A) content estimated from the standard curve.

Length of Poly(A) Tails. The 3′ ends of the poly(A+) RNA population were labeled by successive periodate oxidation and [^{3}H]$NaBH_4$ reduction (Monahan et al., 1976). After RNase A and RNase T_1 digestion, the lengths of the nuclease-resistant poly(A) tails were estimated by electrophoresis on 12% polyacrylamide gels (Monahan & Hall, 1974). Poly(A) standards of known nucleotide length, purchased from Miles Laboratories, were end labeled and subjected to electrophoresis on paralled gels.

Synthesis of cDNA. Highly purified RNA-dependent DNA polymerase from avian myeloblastosis virus was kindly provided by Dr. J. W. Beard (Life Science, Inc., St. Petersburg, FL). The purification procedure was a modification of that of Kacian & Spiegelman (1974), which includes DEAE-cellulose and two phosphocellulose steps. The specific activity of the enzyme was over 10000 units/mg of protein.

Template RNA, 50 μg/mL, was incubated in a 1.2-mL reaction mixture of 50 mM Tris-HCl, pH 8.3, 25 μg/mL oligo(dT) (Collaborative Research, Inc., Waltham, MA), 20 mM dithiothreitol, 8.5 mM magnesium acetate, 20 μg/mL actinomycin D, 50 mM KCl, 450 mM each of dGTP, dATP, and dTTP; 100 μM [^{3}H]dCTP (22.1 Ci/mol; ICN, Irvine, CA), and 400 units/mL RNA-dependent DNA polymerase. The reaction mixture was incubated at 46 °C for 20 min and cDNA purified as previously described (Kuo et al., 1976) by alkali treatment and passage through a Sephacryl-200 column. The cDNA chain length was 950 nucleotides, and the specific activity was calculated to be 3.7×10^7 dpm/μg.

Plasmid DNA Isolation. Plasmid pI19 was grown in *Escherichia coli* HB101 as described by Norgard et al. (1979). The cells were harvested and lysed with Sarkosyl, and a cleared lysate prepared as described by Clewell & Helinski (1970). Plasmid DNA was purified by CsCl–ethidium bromide density gradients.

cDNA–RNA and cDNA–DNA Hybridizations. Hybridization reactions 3–5 μL in volume were carried out in the presence of 50% formamide, 0.6 M NaCl, 0.01 M Hepes, 0.002 M EDTA, and 0.03% Sarkosyl, pH 7.0, at 43 °C in sealed capillary tubes. In plasmid DNA driven hybridization experiments, pI19 DNA was sonicated to ~300 nucleotides in length prior to use in hybridization experiments. After hybridization, the tubes were stored at −20 °C until analysis.

Table I: Size of Fetus at Different Developmental Stages

trimester	days of gestation	length of fetus (C-R) (cm)	weight of fetus
first	0–90	1–17	0.5–500 g
second	90–180	20–60	0.5–10 kg
third	180–270	60–100	10–50 kg

Table II: Messenger RNA Content of Bovine Pancreas during Fetal Development[a]

developmental stage	% poly(rA) present in poly(A+) RNA (a)	% poly(rA) present in total RNA (b)	% poly(A$^+$) RNA present in total RNA (b/a)
first trimester	3.80	0.039	1.03
second trimester	4.04	0.040	0.99
third trimester	4.36	0.049	1.12

[a] The amount of poly(rA) present in the RNA samples was estimated by hybridization with [^{3}H]poly (dT). The probe was hybridized with known quantities of the various RNA samples. After digestion with S_1 nuclease, the amount of poly(rA) present in the RNA samples was determined from a standard curve produced by the hybridization of [^{3}H]poly(dT) with poly(rA) standards.

For duplex formation samples were treated with S_1 nuclease for 30 min in a buffer containing 0.1 M sodium acetate, pH 4.5, 0.2 M NaCl, and 1.25 mM $ZnSO_4$, 10 μg/mL heat-denatured calf thymus DNA, and 6000 units of S_1 nuclease (Miles Laboratories). When radioactive pI19 DNA was annealed with sonicated unlabeled pI19 DNA, 80% of the radioactivity became S_1 resistant at $C_0t = 1.0$.

Computerized pseudo-first-order kinetic curves were generated from the RNA-driven cDNA hybridization data by using a program designed by Pearson et al. (1977).

Results

Isolation of Poly(A+) RNA from First, Second, and Third Trimester Bovine Fetal Pancreas. The developmental stages of the fetuses were obtained from their length and weight (Table I) (Roberts, 1971). Poly(A+) RNA was isolated from pooled pancreatic total RNA either first, second, or third trimester fetuses by two passages over an oligo(dT)–cellulose column. Comparison of the optical density of the oligo(dT)-bound and -unbound material indicated that approximately 1% of the total RNA bound to the column.

Examination of Poly(A) Tails of Poly(A+) mRNA. The poly(A) content of each preparation of poly(A+) RNA was determined (Table II), and regardless of the stage of development, each of the poly(A+) RNA populations contained approximately 4% poly(A). When this value is compared to the poly(A) content of the total RNA, it is apparent that the mRNA represents about 1% of the total RNA population. This value is in good agreement with the oligo(dT)–cellulose chromatography profile. The poly(A) length for each of the three poly(A+) RNA populations was determined by electrophoresis of labeled poly(A) segments along with poly(A) standards of known length. The poly(A+) RNA isolated from first, second, or third trimester RNA possessed poly(A+) tracts of ~55 nucleotides at their 3′ ends. Since the poly(A) content is known (4%), the mean length of the poly(A+) RNAs is therefore approximately 1350 nucleotides.

Electrophoretic Analysis and Immunoprecipitation of Translation Products of Poly(A+) RNA. The mRNA isolated from the pancreas at various stages of development was tested for its ability to support protein synthesis in a cell-free

Table III: Translation of Poly(A+) RNA from Developmental Stages of Fetal Bovine Pancreas[a]

source of RNA	total Cl_3CCO_2H-insoluble radioactivity (CPM)	net cpm above background (*c*)	immunoprecipitable cpm		% immunoreactive insulin[b]
			(–) insulin (*a*)	(+) insulin (*b*)	
no RNA added	15 312 ± 100				
first trimester pancreas	214 980 ± 1540	199 668	25044 ± 720	4677 ± 220	10.2 ± 0.70
second trimester pancreas	190 325 ± 2740	175 013	26632 ± 830	6855 ± 500	11.3 ± 1.3
third trimester pancreas	76 525 ± 1450	60 713	11933 ± 510	6058 ± 660	9.7 ± 1.0

[a] Poly(A^+) RNA (1.2 μg) isolated from the pancreases of first, second, and third trimester fetuses, respectively, was added to a wheat germ translation system. After incubation, aliquots of the [^{3}H]leucine-labeled translation products were removed for trichloroacetic acid precipitation and immunoprecipitation with antisera against bovine insulin. Each data point represents the average of four determinations ± standard error of the mean. [b] Percent immunoprecipitable insulin = $[(a - b)/c] \times 100\%$.

Table IV: Hybridization of [^{3}H]cDNA to Total RNA

trimester of development		observed $R_0t_{1/2}$	corrected $R_0t_{1/2}$ (0.01)	complexity[a] (nucleotides)	number of mRNA species[b]
cDNA	RNA				
first	first	6800	68	3.4×10^7	2.2×10^4
first	second	4600	46	NA[c]	NA
first	third	2200	22	NA	NA
second	second	680	6.8	3.4×10^6	2.2×10^3
third	first	830	8.3	NA	NA
third	third	2400	24	1.2×10^7	8×10^3

[a] Complexity = $K(R_0t_{1/2})$. $K \cong 5.0 \times 10^5$ (liters × nucleotide pairs/mole of nucleotides × seconds). [b] Assuming an average length of 1500 nucleotides. [c] NA = not applicable.

protein-synthesizing system. When added to a rabbit reticulocyte system, the mRNAs isolated from first and second trimester fetal pancreata were similar in their abilities to stimulate protein synthesis. Messenger RNA isolated from third trimester fetal pancreata, however, was only about 40% as efficient (data not shown). This decreased ability to direct protein synthesis may be due to the degradation of the RNA as was observed in gel electrophoretic profiles. When polypeptides synthesized in the rabbit reticulocyte system were displayed (Figure 1) on the $NaDodSO_4$–polyacrylamide slab gel, qualitatively similar profiles with 9–11 major polypeptide bands were obtained for the in vitro translation products of mRNAs from first, second, and third trimester fetal pancreata.

The same relative translation efficiencies are observed if the mRNA is added to a wheat germ cell-free system (Table III) as compared to a rabbit reticulocyte system. The amount of insulin synthesized in vitro was determined by double-antibody precipitation. Approximately 10% of the total translation products synthesized in response to mRNA derived from each stage of development is specifically immunoprecipitated by insulin antisera.

Complexity of First, Second, and Third Trimester Fetuses. Poly(A+) RNA from first, second, and third trimester fetuses were used as templates for cDNA synthesis by using avian myeloblastosis virus reverse transcriptase. The cDNA was then used in RNA hybridization with total RNA. When each of the total RNA samples (first, second, and third trimester) was hybridized to its homologous cDNA templates (Figures 2–4), the kinetics and extents of hybridization were similar in each case, with the range of each reaction extending over >4 log units.

The $R_0t_{1/2}$ for the hybridization of total RNA from first trimester with cDNA to first trimester poly(A+) RNA (Figure 2) was found to be 6.8×10^3 M·s. The $R_0t_{1/2}$ values for hybridization of second and third trimester RNA to this cDNA were 4.6×10^3 and 2.2×10^3 M·s, respectively (Table IV). The extent of hybridization for second trimester RNA back to first trimester cDNA was 93% of that found when first trimester total RNA was hybridized to first trimester cDNA, while the extent for third trimester was 65% of that for first trimester. The moderate differences in the hybridization kinetics indicate substantial homology in the sequences present in the first, second, and third trimesters. Second trimester

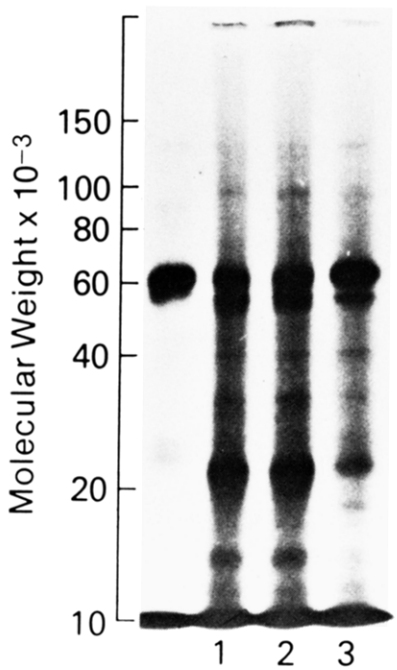

FIGURE 1: $NaDodSO_4$–polyacrylamide electrophoresis of pancreatic mRNA translation products. Poly(A+) RNA was isolated from total RNA (prepared by the phenol–Sevag procedure) followed by two passages over oligo(dT)–cellulose. Poly(A+) RNA (0.5 μg) from the pancreases of first, second, and third trimester fetuses was added to a rabbit reticylocyte translation system, and the resulting [^{35}S]-methionine-labeled translation products were subjected to electrophoresis on an $NaDodSO_4$–polyacrylamide slab gel. Autoradiography of the dried gel revealed the polypeptides synthesized (left to right) in the absence of RNA and in the presence of poly(A+) RNA from the pancreases of first, second, and third trimester fetuses, respectively.

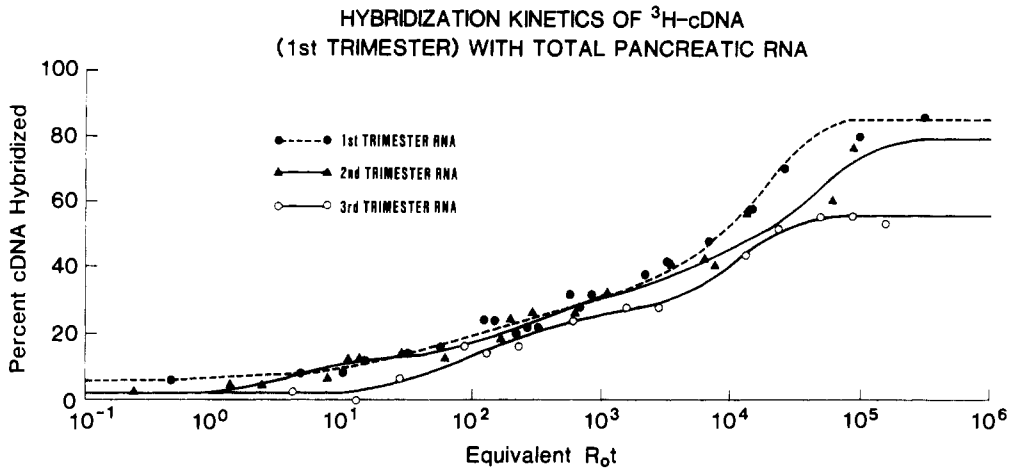

FIGURE 2: Hybridization kinetics of [^{3}H]cDNA (first trimester) with total pancreatic RNA. Total RNA from first trimester (●), second trimester (▲), and third trimester (○) fetuses was hybridized to [^{3}H]cDNA transcribed from total poly(A+) mRNA from first trimester fetuses. Hybridizations were performed in 0.6 M NaCl, 0.01 M Hepes, 0.002 M EDTA, and 0.03% Sarkosyl at 68 °C, in sealed 5-μL capillary tubes.

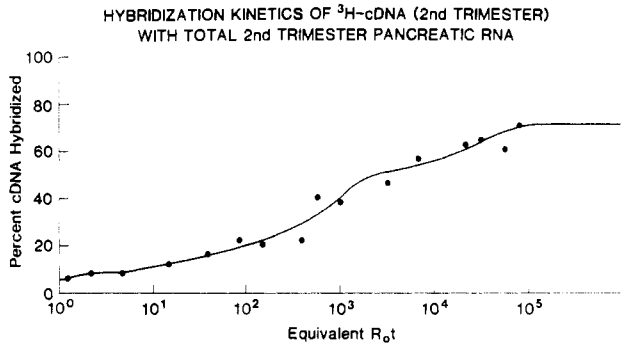

FIGURE 3: Hybridization kinetics of [^{3}H]cDNA (second trimester) with total second trimester pancreatic RNA. RNA-driven hybridization of total RNA from second trimester fetal pancreata, with cDNA transcribed from second trimester pancreatic poly(A+) RNA. Hybridizations were performed in 0.6 M NaCl, 0.01 M Hepes, 0.002 M EDTA, and 0.03% Sarkosyl at 68 °C in 5-μL sealed capillary tubes. Hybrid formation was estimated by the S_1 nuclease assay.

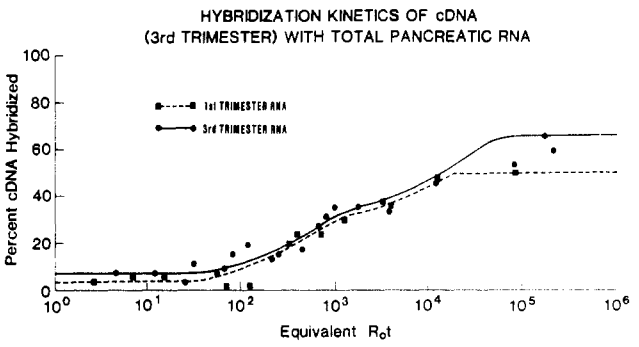

FIGURE 4: Hybridization kinetics of [^{3}H]cDNA (third trimester) with total pancreatic RNA. RNA-driven hybridization of total RNA from first and third trimester fetal pancreas with cDNA transcribed from third trimester pancreatic poly(A+) RNA. The hybridization was performed in 0.6 M NaCl, 0.01 M Hepes, 0.002 M EDTA, and 0.03% Sarkosyl at 68 °C, in sealed 5-μL capillary tubes, and hybrid formation was estimated by the S_1 nuclease assay.

RNA hybridized to second trimester cDNA, with a $R_0t_{1/2}$ of 6.8×10^2 M·s (Figure 3).

Third trimester total RNA hybridized to its homologous cDNA with a $R_0t_{1/2}$ of 2.4×10^3 M·s (Figure 4), while the $R_0t_{1/2}$ for the hybridization of first trimester total RNA back to third trimester cDNA was 8.3×10^2 M·s; the extent of hybridization was 77% of the extent of hybridization between third trimester total RNA and cDNA to third trimester poly(A+) RNA, and, as in the reciprocal experiment, once again the differences in the kinetics of hybridization were more apparent in the high complexity component.

Table V: Hybridization of Plasmid pI19 DNA with cDNA to Poly(A+) RNA from First, Second, and Third Trimester RNA

developmental stage	% cDNA hybridized to plasmid	% insulin mRNA by weight[a]
first trimester	5.1	7.6
second trimester	8.6	12.9
third trimester	2.5	3.9

[a] Based on an estimated 600 nucleotide length of the insulin mRNA.

Hybridization of pI19 DNA to cDNA. Insulin gene expression was estimated by nucleic acid hybridization. Rat plasmid pI19 containing rat preproinsulin I sequences (Villa-Komaroff et al., 1978) was sonicated to 300 base pairs in length and hybridized to [^{3}H]cDNA transcripts of poly(A+) RNA of each trimester of fetal development. Since the experiments were carried out at excess plasmid DNA, all complementary sequences in the cDNA should have been hybridized to the plasmid DNA by $C_0t = 50$. In the first trimester of development, 5.1% of the cDNA transcribed from poly(A+) RNA is homologous to pI19 (Table V). This value increases to 8.6% during the second trimester and then decreases to 2.5% in the third trimester. These values represent the percentage of cDNA homologous to only 400 nucleotides of the insulin message since the full message sequence is not present in the insert of pI19. Assuming a length of approximately 600 nucleotides for the total insulin message, then the corrected percentages of cDNA that is representative of insulin mRNA are 7.6%, 12.9%, and 3.9% for first, second, and third trimester poly(A+) RNA, respectively.

The cross reaction of rat insulin sequences with bovine DNA has been demonstrated by Southern blotting. Bovine DNA was digested with restriction endonuclease enzyme *Hin*dIII and *Eco*RI. These single digests were fractionated on agarose gels, transferred to nitrocellulose paper, hybridized with ^{32}P-labeled pI19 DNA, and the hybrids detected by autoradiography. A single band at 3.8 kb was detected in the *Hin*dIII digest, and three bands at 7.8, 5.6, and 4.2 kb were found in the *Eco*RI digests (data not shown). These experiments indicate specific hybridization of the rat insulin plasmid with homologous sequences in bovine DNA.

Discussion

RNA isolated from first, second, and third trimester bovine fetal pancreas was used to study the development of the pancreas. The content and length of the poly(A) tails from

the poly(A+) RNA were the same for all three trimesters, suggesting that the mean length of the poly(A+) RNAs was the same (1350 nucleotides) in all three cases. The cDNA transcripts of each of the three RNA populations were also of the same average length, making interpretation of hybridization experiments less complicated.

From the kinetics of hybridization of total cellular RNA from the three developmental stages with cDNAs transcribed from poly(A+) RNA of their respective stage of development, we hoped to gain some insight into the extent of differential gene expression during development. At the same time we were able to examine the heterogeneity of each of the individual RNA populations.

The results of such hybridization experiments demonstrate that the poly(A+) RNA populations from each stage of development are very heterogeneous and complex. This is to be contrasted with the adult rat pancreatic poly(A+) RNA population in which a similar hybridization reaction took place over two log units (Harding et al., 1977). Changes in the amount of RNA complementary to the first trimester cDNA could be detected when total RNA populations from first, second, and third trimesters were compared. The extent of hybridization of second and third trimester RNA with first trimester cDNA were 93% and 65%, respectively, of that found in hybridization of first trimester RNA with first trimester cDNA; therefore 7% of the first trimester mRNA is no longer present at detectable levels in the second trimester, and by the third trimester, 35% of the first trimester mRNA is no longer present at detectable levels. These changes represent qualitative changes during fetal development. The extent of hybridization of first trimester RNA with third trimester cDNA was 77% of the extent of hybridization between third trimester RNA and third trimester cDNA, suggesting that 23% of the poly(A+) RNA present during the third trimester had not yet appeared in the RNA population during the first trimester.

The hybridization experiments are summarized in Table IV. The complexities of the three RNA populations were 3.4×10^7, 3.4×10^6, and 1.2×10^5 nucleotides for first, second, and third trimester, respectively. Assuming an average of 1500 nucleotides per mRNA molecule, there would be 2.2×10^4 mRNA species in the first trimester, 2.2×10^3 in the second trimester, and 8×10^3 in the third trimester. The profiles from gel electrophoresis of the in vitro translation products of poly(A+) RNA are in agreement with results from RNA-driven hybridization experiments. The translation products from poly(A+) RNA of all three trimesters are heterogeneous, and very few qualitative changes are observed during the progression from one developmental stage to the next.

We have estimated the concentration of insulin mRNA at each stage of development by immunoprecipitation of translation products obtained from in vitro translation of the poly(A+) RNA populations and by hybridization of pI19, a plasmid containing 400 nucleotides of the sequence for rat preproinsulin I mRNA, with cDNA synthesized from the three poly(A+) RNA populations. The results of immunoprecipitation data (Table III) suggest that changes occurring in the concentration of the insulin mRNA are not as pronounced as predicted by nucleic acid hybridization (Table V). Since it has been demonstrated that the determination of percent IRI in populations of in vitro translation products are extremely sensitive to changes in the Mg^{2+}, K^+, and mRNA concentrations (Lomedico & Saunders, 1977) as well as degradation, the hybridization data may more accurately reflect the concentration of the insulin RNA sequences. Another possible explanation for the discrepancies between the translation and hybridization assays is that there could be two mRNA species coding for the insulin mRNA. If our molecular hybridization probe hybridized with only one of the mRNA species, while the immunoprecipitation technique could detect translation products from both mRNA species, then these results might be obtained. This possibility seems unlikely because of the great similarity in nucleotide sequences among insulin genes in the rat and other mammals.

Since we are dealing with an organ and not a pure cell line, it is difficult to determine what control mechanisms are regulating insulin mRNA levels. An initial increase in insulin mRNA concentration from first to second trimester may reflect a faster rate of β cell development relative to the total mass of the pancreas. The decrease in the insulin mRNA concentration as the fetus progresses from the second trimester to the third trimester may result from dilution of the endocrine cells by the exocrine cells, since the Acinar cells comprise larger proportions of the pancreatic mass during the later developmental progression of the bovine fetus (Weir & Like, 1975).

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