Changes in the Frequency of Specific Transcripts during Development of the Pancreas*

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The sequence complexity and frequency distribution of adult rat pancreas polyadenylated RNAs and the changes in these pancreas transcripts during development have been analyzed by means of complementary DNA-RNA hybridization analysis. The following results were obtained. (a) An abundant set of pancreas polyadenylated RNAs hybridizes with 90% of pancreas cDNA (the copy of adult rat pancreas polyadenylated RNA), comprises about 2% of total pancreas RNA, has a sequence complexity of about $2 \times 10^4$ nucleotides, and is largely tissue specific. These and other data imply that these RNAs code primarily for pancreas-specific secretory proteins. (b) A less abundant set of pancreas RNAs hybridizes with HTC cell cDNA (the copy of HTC cell polyadenylated RNA), comprises about 0.03% of total pancreas RNA, has a sequence complexity of at least $3 \times 10^5$ nucleotides, and is more frequent in rapidly proliferating tissues (HTC cells and embryonic pancreas) than in the adult pancreas. (c) In contrast to the pancreas, the relatively undifferentiated HTC cell does not contain a predominant set of polyadenylated RNAs of low complexity. (d) Moderate concentrations of RNAs complementary to pancreas cDNA are present in pancreatic rudiments at 14 days of gestation when levels of pancreas-specific proteins are low. Between 14 and 20 days of gestation, the concentration of these RNAs increases several hundred-fold in the embryonic pancreas, in parallel with the increased rate of synthesis of pancreas-specific proteins. (e) Pancreas cDNA hybridizes in situ primarily to the regions of acinar cells actively synthesizing secretory proteins. The very favorable signal-to-noise ratio of the technique indicates that it will be useful for detecting low amounts of pancreas-specific RNAs early in development.

Tissue-specific gene products accumulate in a biphasic pattern during the embryonic development of the rat pancreas (1, 2). 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 thousand-fold as a consequence of a substantial (at least 100-fold) increase in their rates of synthesis. This synthetic program can arise from modulation of the rate of transcription of specific genes, post-transcriptional regulation of mRNA function, or both. Since the RNAs coding for the synthesis of pancreatic exocrine proteins are polyadenylated (3, 4), these modes of regulation can be distinguished in part by an analysis of the variation in concentration of pancreatic polyadenylated RNAs during development.

In the initial studies, reported here, we examine the distribution of RNA sequences in adult rat pancreas polyadenylated RNA and follow changes in the concentration of specific pancreatic RNAs during development by means of cDNA-RNA hybridization analysis. The kinetics of hybridization of a cDNA with an excess of the RNA from which it is copied reflects the sequence complexity (total length of different RNA sequences) of the RNA preparation and the relative frequency of various species within it (5). The tissue specificity of the RNA is determined by hybridizing its cDNA with RNA isolated from other tissues. We find that 90% of the cDNA synthesized from pancreas polyadenylated RNA (pancreas cDNA) is complementary to a relatively small number of RNA species present in high steady state concentrations in the differentiating pancreas. In contrast, the RNA isolated from a rat tumor cell line (HTC) which is not obviously differentiated does not contain a predominant low complexity component. We find, in addition, that most of the pancreas cDNA does not hybridize with RNA isolated from other tissues. We therefore have used pancreas cDNA as a probe to detect changes in the concentrations of pancreas-specific polyadenylated RNAs during development.

MATERIALS AND METHODS

Isolation of RNA—All glassware and utensils were freed of ribonuclease contamination either by heating for 9 h at 180° or treating with 0.2% diethylpyrocarbonate for 1 h and thoroughly washing with sterile water. All solutions were autoclaved or treated with 0.2% diethylpyrocarbonate before use. The following procedure was used for the isolation of RNA from 1 g of adult rat pancreas or
other tissue (6, 7). Freshly isolated tissue was homogenized in 20 ml of 5.7 M guanidine hydrochloride, 1% diethylpyrocarbonate, 0.1 M potassium acetate, pH 5, with a Tissumizer (Tekmar Industries, Cincinnati, Ohio) homogenizer at top speed for 1/2 min. One-half volume of 50% ethanol was added and the solution placed on ice for 10 min. The precipitate, which contains the RNA, was collected by centrifugation at 4000 x g for 10 min and dissolved at room temperature in 10 ml of 5.7 M guanidine hydrochloride, 25 mM EDTA, pH 7. Then 0.5 ml of 2 M potassium acetate, pH 5, and 5 ml of 95% ethanol were added and the solution incubated at -20°C for 1 h. The precipitating RNA was then collected, redissolved, and precipitated as before. The final RNA precipitate was dissolved in 5 ml of 20 mM EDTA, pH 7, and shaken with 10 ml of water-saturated CHCl3:iso-octane (4:1, v/v). After centrifugation (4000 x g, 10 min), the aqueous phase was removed and the organic phase re-extracted twice with 3 ml of 20 mM EDTA, pH 7. Two volumes of 4.5 M potassium acetate, pH 6, were added to the pooled aqueous phases and the RNA was precipitated overnight at -20°C (8). The RNA was pelleted, dissolved in water, made 0.2 M in potassium acetate, pH 5, and precipitated by the addition of 2.5 volumes of 95% ethanol.

Oligo(dT)-Cellulose Chromatography—Total RNA was passed over an oligo(dT)-cellulose column (Collaborative Research, Inc., type 2) in 0.5 M KCl, 10 mM Tris/HCl, pH 7.8. The bound RNA was eluted with 0.5 M KCl, 10 mM Tris/HCl, pH 7.6. The unbound RNA was precipitated by the addition of 2.5 volumes of ethanol and 10% sodium acetate, pH 4.5, and precipitated by the addition of 2.5 volumes of 1 M sodium acetate, pH 4.5, 0.3 M NaCl, 3 mm ZnCl2, 10 µg/ml of denatured calf thymus DNA. This mixture was divided into an aliquot (0.66 µl to which was added 0.5 µl of 32P-labeled mouse DNA marker (56). The acid-precipitable nucleic acid was collected on Whatman GF/C filters, washed with 5% trichloroacetic acid containing 0.01 M sodium pyrophosphate and dried. The radioactivity was determined by liquid scintillation counting. The fraction of hybridized cDNA was determined by comparing the acid-precipitable radioactivity in the aliquot of each reaction to that of the aliquot of each reaction as determined by hybridization to S1 nuclease to the radioactivity of the undigested aliquot.

In Situ Hybridization—In situ hybridization was performed by the method of Harrison et al. (14) modified for use with sectioned tissue. Small pieces of adult rat pancreas were frozen in liquid nitrogen and sectioned in a cryostat (International) at -10°C. Sections (5 to 7 µm thick) were collected on glass slides coated with chrome alun (0.1%)/gelatin (1%), fixed in acetone/methanol/acetone (v/v), and rinsed in 2 x SSC. Pancreas cDNA (200,000 cpm in 5 to 10 µl of 3 x SSC containing 40% formamide) was pipetted onto the section, covered with a glass coverslip, and incubated at 45°C overnight. The coverslip was removed, the slide was washed in 2 x SSC, and incubated for 90 min at 55°C in 2 x SSC. The slide was dried, coated with Hiford L-4 emulsion, and exposed for 3 days to 3 weeks.

Electrophoretic Analysis of RNA—Cylindrical polyacrylamide gels containing 99% formamide and 0.5% acrylamide were prepared and run by the method of Pinder et al. (10). Following electrophoresis, the gels were soaked overnight in 5% acetic acid containing 1% lanthanum acetate and scanned at 260 nm.

Sequence Heterogeneity of Adult Pancreas Polyadenylated RNA

Isolation of Pancreas RNA and Synthesis of Pancreas cDNA—Intact RNA was readily isolated from adult rat pancreas by the guanidine HCl/diethylpyrocarbonate procedure outlined under "Materials and Methods" despite high levels of endogenous ribonuclease. The yield of RNA is about 22 mg/g of pancreatic tissue. The electrophoretic profile of total adult rat pancreas RNA run on denaturing polyacrylamide gels containing 99% formamide is shown in Fig. 1A. The 28 S and 18 S ribosomal RNAs are present in about a 2:1 ratio.

The abbreviation used is: SSC, 0.15 M NaCl, 15 mM sodium citrate, pH 7.

The data were analyzed by nonlinear least squares regression analysis using the equation (G. Swift and B. J. McCarthy, unpublished)

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H_{\text{Dreal}} = \sum_{i=1}^{n} H_i \left[ \frac{D}{\left(1 - \frac{R_o}{R_i}\right)^{1/4}} \right] \]

for n classes of RNA: \(H_{\text{Dreal}} = \) cDNA hybridized/total cDNA; \(H_i / D_i = \) cDNA of complexity class i/total cDNA; \(H_i / D_i = \) assumed to equal \(R_i / K_{i} = \) RNA of class i/total RNA; \(R_i / K_{i} = \) value of complexity class i; \(R_0 / K_{i} = \) value of a given class diploid.
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The RNA preparation is relatively free of low molecular weight (4 to 5 S) species that can be precipitated from the initial guanidine HCl/diethylpyrocarbonate homogenate by higher concentrations of ethanol than are used here. The RNA contains less than 0.5% DNA when assayed by the diphenylamine reaction. The electrophoretic profile of pancreas oligo(dT)-bound RNA is shown in Fig. 1B. Two major peaks of absorbance are present, both of which code for the in vitro synthesis of polypeptides which we infer to be precursors of pancreatic exocrine proteins. Most of the intact 28 S RNA is removed by the column procedure, but some 18 S RNA is present. The oligo(dT)-bound RNA contains about 40% polyadenylated RNA having an average poly(A) length of 60 nucleotides (data not shown).

Oligo(dT)-bound pancreas RNA was copied with viral RNA-dependent DNA polymerase. The reaction required an oligo(dT) primer, indicating that polyadenylated RNA was copied. The cDNA product was deproteinized, freed of RNA by alkaline hydrolysis, and fractionated on an alkaline sucrose gradient. The cDNA sedimenting faster than 6 S (approximately two-thirds of the preparation) was pooled and employed in the hybridization experiments reported below. It is henceforth designated pancreas cDNA. The average size of pancreas cDNA is 650 nucleotides determined by formamide gel electrophoresis with phage λ DNA EcoRI + HindIII restriction fragments as standards. The cDNA sedimenting slower than 6 S was not used in these experiments since about 40% of it did not hybridize with pancreas RNA at high $R_{t}$ values, suggesting that small cDNAs do not form stable hybrids in our incubation and assay conditions. A similar observation has been made by others (20, 21).

**Hybridization of Pancreas cDNA with Pancreas RNA**

The kinetics of hybridization of pancreas cDNA with an excess of the template RNA preparation (oligo(dT)-bound RNA) are shown in Fig. 2. About 90% of the cDNA hybridized between $R_{t}$ values of $5 \times 10^{-4}$ and $5 \times 10^{-1} \text{ mol s liter}^{-1}$. This range of $R_{t}$ values is greater than that expected for the hybridization of a single RNA species with its cDNA. Computer analysis indicates that a hybridization curve containing two ideal pseudo-first order kinetic components provides a good fit to the data. The first component of the curve comprises about 0 to 50% hybridization, has an observed $R_{t}$ of $5.1 \times 10^{-4} \text{ mol s liter}^{-1}$, and a complexity of about 1.7 \times 10^5 nucleotides (Table I). The second component comprises about 50 to 90% hybridization, has an observed $R_{t}$ of $7.4 \times 10^{-4} \text{ mol s liter}^{-1}$, and a complexity of about 1.9 \times 10^5 nucleotides. The RNA that hybridizes with 90% of pancreas cDNA therefore has a total complexity of about $2 \times 10^5$ nucleotides and is most simply viewed as comprising two sequence populations present in different concentrations in the tissue. Clearly, however, curves containing more components can also be fit to the data. Oligo(dT)-bound RNA hybridized with about 95% of pancreas cDNA at a $R_{t}$ value of $10^{-3} \text{ mol s liter}^{-1}$ (data not shown), implying that a more diverse and less abundant class of RNAs was also copied. The increment of hybridization above the 90% level shown in Fig. 2 is just greater than the experimental error of duplicate determinations. Therefore we have used a heterologous (HTC cell) cDNA to unambiguously demonstrate the existence of a high complexity class of pancreatic RNA sequences (see below).

The kinetics of hybridization of pancreas cDNA with total adult rat pancreas RNA are shown in Fig. 2 (closed circles). Comparison of the $R_{t}$ value of the reaction with that of the oligo(dT)-bound RNA hybridization (see legend to Fig. 2)
60% of the cDNA hybridized between RNA. The kinetics of hybridization of HTC cell cDNA and Total adult pancreas RNA hybridizes to about 40% of HTC cell cDNA at a $R_{4/12}$ value of 1.6. The tissue specificity of pancreas cDNA was assessed by hybridization with RNA isolated from other rat tissues. Two hybridization curves are shown in Fig. 2 and the results are summarized in Table II. Total rat liver, brain, submaxillary gland, and HTC cell (a rat tumor cell line of hepatic origin) RNAs hybridize to 10 to 22% of pancreas cDNA at a $R_{4/12}$ value of $10^2$ mol s liter$^{-1}$ at which an apparent plateau of hybridization is obtained. These results do not reflect DNA contamination of the RNA preparations, since the hybridizable material is alkali labile. The addition of brain RNA to submaxillary gland, HTC cell, or liver RNA does not increase the amount of cDNA hybridized, indicating that a set of RNA sequences complementary to pancreas cDNA is common to all of the tissues. Therefore, about 80% of pancreas cDNA is a copy of pancreas-specific RNA species within the limitations of the analysis.

Hybridization of HTC Cell cDNA with Other Rat Tissue RNAs — The tissue specificity of pancreas cDNA was assessed by hybridization with RNA isolated from other rat tissues. The minimum complexity of the pancreas RNA that hybridizes with HTC cell cDNA can be estimated from this result and the data in Table I. The first two components of the HTC cell hybridization curve have a total complexity of about 2.9 x 10$^2$ nucleotides and comprise about 80% of pancreas RNA. Therefore, about 80% of pancreas cDNA is a copy of pancreas-specific RNA species within the limitations of the analysis.

Hybridization of HTC Cell cDNA with HTC Cell RNA — A heterologous cDNA, useful for examining pancreas RNA populations, was synthesized from HTC cell oligo(dT)-bound RNA. The kinetics of hybridization of HTC cell cDNA and HTC cell oligo(dT)-bound RNA are shown in Fig. 3. About 60% of the cDNA hybridized between $R_{4/12}$ values of $10^2$ and $10^3$ mol s liter$^{-1}$, implying that a heterogeneous RNA population was copied. For computational purposes, we have fit a three component hybridization curve to the data although higher order curves can also be used. The sequence complexity of each of the component was calculated (Table I). The data indicate that one frequency class of HTC cell polyadenylated RNA has a complexity as low as $5.4 \times 10^2$ nucleotides and another class has a complexity as great as $6.6 \times 10^2$ nucleotides. Since only 60% of the cDNA was hybridized at the highest $R_{4/12}$ value tested, a component of even higher complexity has probably been copied but not hybridized.

Hybridization of HTC Cell cDNA with Pancreas RNA — Total adult pancreas RNA hybridizes to about 40% of HTC cell cDNA at a $R_{4/12}$ value of $1.6 \times 10^3$ mol s liter$^{-1}$ (Fig. 3). The minimum complexity of the pancreas RNA that hybridizes with HTC cell cDNA can be estimated from this result and the data in Table I. The first two components of the HTC cell hybridization curve have a total complexity of about $2.9 \times 10^2$ nucleotides and comprise about 40% of the cDNA. If pancreas RNA hybridizes only to these cDNA sequences, it has a complexity of at least $2.9 \times 10^2$ nucleotides, which is 10 times greater than the complexity of pancreas polyadenylated RNA calculated from Fig. 1. This is a minimal estimate since pancreas RNA may hybridize to third component HTC cell cDNA and therefore could have a complexity at least 10 times greater than this value.

That the pancreas RNA sequences complementary to HTC cell cDNA comprise a minor fraction of pancreas RNA is indicated by further analysis of the data in Fig. 3. Comparison of the $R_{4/12}$ values of the reactions (see legend, Fig. 3) indicates that polyadenylated RNAs complementary to pancreas cDNA comprise about 2% of total pancreas RNA.

**Table I**

| Tissue                  | Component | Fraction of cDNA hybridized | $R_{4/12}$ | Complexity of component | Total complexity |
|-------------------------|-----------|-----------------------------|------------|-------------------------|-----------------|
|                         | 1         | 51                          | 5.1 x 10$^{-3}$ | 1.0 x 10$^{-3}$ | 1.7 x 10$^{3}$ | 2.1 x 10$^{4}$ |
|                         | 2         | 38                          | 7.4 x 10$^{-2}$ | 1.1 x 10$^{2}$ | 1.9 x 10$^{3}$ | 3.9 x 10$^{4}$ |

**Table II**

| Extent of hybridization of rat pancreas cDNA with rat tissue RNAs | cDNA hybridized | % |
|------------------------------------------------------------------|-----------------|---|
| Pancreas                                                         | 92              |   |
| Brain                                                            | 13              |   |
| Submaxillary gland                                               | 12              |   |
| HTC cell                                                         | 10              |   |
| Submaxillary + brain                                             | 13              |   |
| HTC cell + brain                                                 | 13              |   |
| Liver                                                            | 22              |   |
| Liver + brain                                                   | 24              |   |

* Rat pancreas cDNA was hybridized with total RNA isolated from a given tissue to a $R_{4/12}$ value of $10^3$ mol s liter$^{-1}$.
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indicates that about 1.5% of total HTC cell RNA and about 0.03% of total pancreas RNA are complementary to HTC cell cDNA, respectively. Although these calculations are rather inexact due to the complex shapes of the curves, the latter value is clearly much less than the percentage of total pancreas RNA complementary to pancreas cDNA (about 2%, see above). If it is assumed that the pancreatic RNA species complementary to HTC cell cDNA are polyadenylated, they maximally comprise about 2% of pancreas polyadenylated RNA.

Cellular Distribution of Abundant Pancreas Polyadenylated RNAs

The distribution of specific transcripts among different pancreatic cell types was examined by means of in situ hybridization. Pancreas cDNA was incubated with fixed tissue sections of adult pancreas and hybrid structures visualized by autoradiography (Fig. 4). The acinar tissue is very heavily labeled, indicating that the cDNA hybridized extensively in situ. In contrast, the lumen of a blood vessel (b) is unlabeled and the endocrine cells of the islets of Langerhans (i) and connective tissue cells (c) are very weakly labeled. Fig. 5 shows a higher magnification of the autoradiograph. The basal and perinuclear regions of acinar cells are highly labeled. This area contains the majority of the rough endoplasmic reticulum. In contrast, the nuclei (arrows), cell apices, and the lumens of the acini (l) are poorly labeled. Thus pancreas cDNA hybridizes primarily with RNAs present in the regions of acinar cells actively synthesizing secretory proteins.

Changes in the Concentration of Pancreas RNAs during Development

Embryonic Pancreas RNAs Complementary to Pancreas cDNA - In order to determine whether the abundance of RNA species complementary to pancreas cDNA changes during development, RNAs isolated from fetal rat pancreases of increasing gestational age were hybridized with pancreas cDNA. The kinetics of hybridization are shown in Fig. 6. The following results are obtained.

(a) The embryonic hybridization curves shift toward the position of the adult curve on the Rₜ⁺ scale as development proceeds, indicating that the concentration of RNA sequences complementary to pancreas cDNA increases as a function of gestational age. The complex shapes of the hybridization curves in Fig. 6 preclude a precise calculation of changes in RNA concentration. However, the 14- and 16-day hybridization curves are approximately 1 log unit apart on the Rₜ⁺ scale as are the 16- and 18-day curves, implying about a 10-fold increase in the concentration of RNAs complementary to pancreas cDNA during each 2 days of development. The 18- and 20-day curves are somewhat less than 1 unit apart on the Rₜ⁺ scale, indicating a smaller increase in RNA concentration. The concentration of RNA species characteristic of adult pancreas therefore increases about 500-fold between 14 days of development and adulthood.

(b) The maximum amount of hybridization obtained is not the same for all embryonic RNAs. At the highest Rₜ⁺ values tested, adult, 20-, and 18-day RNAs hybridize with about 90% of pancreas cDNA and 16- and 14-day RNAs hybridize with 60 and 30% of the cDNA, respectively.

(c) The embryonic hybridization curves (Fig. 6) are not all the same shape as the adult hybridization curve (Fig. 2). The 20- and 18-day RNAs hybridize over more than 4 orders of magnitude of Rₜ⁺ values whereas adult RNA hybridizes over about 3 orders of magnitude.

(d) The shape of the embryonic hybridization curves differ from each other at relatively high Rₜ⁺ values. There is an obvious transition in the 18-day curve at a Rₜ⁺ value of 10⁶ mol s liter⁻¹ (65% hybridization) that is not evident in the 20-day curve.

Embryonic RNA Complementary to HTC Cell cDNA - The kinetics of hybridization of 18-day embryonic pancreas RNA with HTC cell cDNA is shown in Fig. 3 (closed squares). About 50% of the cDNA hybridizes at a Rₜ⁺ value of 10⁶ mol s
The less abundant pancreas RNAs decrease about 10-fold between 18 days of development and suggests that about 0.4% of total adult pancreas RNA is complementary to HTC cell cDNA as compared with about 80% of pancreas polyadenylated RNA which is complementary to a polyadenylated RNA population of high sequence complexity. Complexity values determined from the kinetics of hybridization of pancreas RNAs, estimated here as $2 \times 10^4$ nucleotides, which is sufficient to code for about 15 mRNA sequences of average size. (f) The low sequence complexity of the majority of pancreas polyadenylated RNA is not an artifact of the isolation procedure since HTC cell RNA, isolated and copied into cDNA by the same techniques, clearly contains a polyadenylated RNA population of high sequence complexity. Furthermore, the result is not unique to the rat pancreas. Undegraded RNA can be isolated from dog pancreas by phenol extraction because the dog, unlike the rat, does not synthesize significant amounts of exportable ribonuclease (31). Our unpublished experiments indicate that the kinetics of hybridization of dog pancreas cDNA with phenol- or guanidine HCl/diethylpyrocarbonate-extracted dog oligo(dT)-bound RNA are similar to the kinetics of hybridization of rat pancreas cDNA-RNA shown here.

Changes in Concentration of Pancreas-specific RNAs during Development—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 during the period of extensive morphogenesis (the
curves are about 2 orders of magnitude apart, implying about 14 and 18 days of gestation. Exact numerical translation of mRNA synthesized in the protodifferentiated state (1, 2, 32). Some polyadenylated RNAs present in high frequency in the adult pancreas are also synthesized in measurable amounts in the protodifferentiated pancreas. Fourteen-day pancreas RNA hybridizes with 10 to 20% more pancreas cDNA at relatively high $R_{tl}$ values than does RNA isolated from nonprotodifferentiated tissues, suggesting that at least some of these RNAs are pancreas-specific. The experiments do not reveal whether the protodifferentiated pancreas synthesizes all or only a subset of pancreas-specific polyadenylated RNAs. The small size of the 14-day pancreatic rudiment limits the amount of RNA that can be isolated and thus the maximum $R_{tl}$ value of the hybridization reaction. It is thus possible that at higher $R_{tl}$ values, 14-day RNA would hybridize with more than 30% of pancreas cDNA.

The second phase of pancreas-specific protein synthesis begins between Days 14 and 15 of gestation and is characterized by a dramatic increase in the rates of synthesis of the pancreatic exocrine proteins concomitant with cytodifferentiation (1, 2, 32). The general features of this developmental period are reflected in the kinetics of hybridization of embryonic pancreas RNAs with pancreas cDNA. First, the rates of synthesis of exocrine proteins increase in a generally synchronized but noncoordinate fashion, resulting in variations in the relative amounts of individual proteins during gestation. Similarly, the embryonic hybridization curves vary somewhat in shape, implying differences in the relative concentration of polyadenylated RNA species during this period. Second, the rate of synthesis of an exocrine protein such as amylase or chymotrypsinogen increases steadily between Days 14 and 20 of gestation and is about 100 times greater at Day 18 than at Day 14. The relative concentration of RNAs complementary to adult pancreas cDNA also increases during development as evidenced by the position of the embryonic RNA hybridization curves on the $R_{tl}$ scale. The 18- and 14-day hybridization curves are about 2 orders of magnitude apart, implying about a 100-fold increase in specific polyadenylated RNA concentration between 14 and 18 days of gestation. Exact numerical values of the concentration of pancreas-specific RNAs cannot be obtained from the data due to the complex shapes of the embryonic hybridization curves. However, the data are consistent with the hypothesis that the increased rate of synthesis of pancreas-specific proteins arises primarily from an increased rate of transcription rather than an increased rate of translation of mRNA synthesized in the protodifferentiated state.

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