

Isolation of an insulin-like growth factor II cDNA with a unique 5' untranslated region from human placenta

(fetal growth/alternative splicing/alternative promoters/specific expression)

SHU-JANE SHEN*, MAKOTO DAIMON*, CHUN-YEH WANG*, MAARTEN JANSEN†, AND JUDITH ILAN*‡§

*Department of Reproductive Biology and †Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, OH 44106; and ‡Department of Pediatrics, State University of Utrecht, Utrecht, The Netherlands

Communicated by L. Lorand, November 16, 1987 (received for review October 6, 1987)

ABSTRACT Human insulin-like growth factor II (IGF-II) cDNA from a placental library was isolated and sequenced. The 5' untranslated region (5'-UTR) sequence of this cDNA differs completely from that of adult human liver and has considerable base sequence identity to the same region of an IGF-II cDNA of a rat liver cell line, BRL-3A. Human placental poly(A)⁺ RNA was probed with either the 5'-UTR of the isolated human placental IGF-II cDNA or the 5'-UTR of the IGF-II cDNA obtained from adult human liver. No transcripts were detected by using the 5'-UTR of the adult liver IGF-II as the probe. In contrast, three transcripts of 6.0, 3.2, and 2.2 kilobases were detected by using the 5'-UTR of the placental IGF-II cDNA as the probe or the probe from the coding sequence. A fourth IGF-II transcript of 4.9 kilobases presumably containing a 5'-UTR consisting of a base sequence dissimilar to that of either IGF-II 5'-UTR was apparent. Therefore, IGF-II transcripts detected may be products of alternative splicing as their 5'-UTR sequence is contained within the human IGF-II gene or they may be a consequence of alternative promoter utilization in placenta.

Insulin-like growth factors I and II (IGF-I and IGF-II) are polypeptide mitogens that share structural homology with insulin and exhibit a similar spectrum of biologic activities (1-4). They are thought to be important in postnatal growth (5, 6). Several observations also suggest that IGF-II may play a role in fetal growth and development (7-10). Expression of human IGF-II mRNA has been examined in various fetal and adult tissues, including tumors (11-13). The cDNA sequences of IGF-I and IGF-II predict that both are synthesized as large precursor molecules that undergo extensive processing. Recently, we reported the expression of the IGF-II gene in human placenta and showed that there are changes in the relative abundance of IGF-II mRNA during gestation (14). Four major transcripts of 6.0, 4.9, 3.2, and 2.2 kilobases (kb) were detected when hybridized with a human liver-derived IGF-II cDNA probe containing the coding region.

Here we describe the isolation and sequencing of IGF-II cDNA from a human placental library.¶ The 5' untranslated region (5'-UTR) sequence of this cDNA differs completely from that reported for human adult liver (15). It has considerable base sequence identity to the same region of IGF-II cDNA isolated from a rat liver cell line, BRL-3A (16), and to a small partial sequence reported recently for IGF-II cDNA isolated from human hepatoma (17). By using the 5'-UTR as probe we demonstrated that placenta expresses no IGF-II transcripts with adult liver-like 5'-UTR and three of the four defined placental IGF-II transcripts hybridize to the placental 5'-UTR probe. These results indicate that the IGF-II gene may undergo an alternative splicing process as both UTR

sequences are contained within the single IGF-II gene (16, 17). Alternatively, it may be a result of tissue-specific promoter utilization and could be of developmental significance or be related to tissue-specific expression. It is also of interest that placental IGF-II transcripts are expressed at a much higher level in diabetic pregnancies.

METHODS

Placentas were handled as described (14). The cDNA library was prepared according to Gubler and Hoffman (18). By using 10 µg of poly(A)⁺ RNA from a term placenta of an overt diabetic (class B), 1 × 10⁵ individual clones were obtained. Colony hybridization was performed by using a ³²P-labeled IGF-II cDNA insert as described (14). The [α-³²P]dCTP-labeled nick-translated probe had a specific activity of 2-5 × 10⁷ cpm/µg of DNA. Hybridization solution consisted of probe at a concentration of 1 × 10⁶ cpm/ml, 0.3 M NaCl/30 mM sodium citrate, 5 × concentrated Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 0.1% sodium pyrophosphate, 100 µg of sonicated/denatured *Escherichia coli* chromosomal DNA per ml, 10% dextran sulfate, and 0.1% sodium lauryl sulfate. Hybridization was performed at 60°C. Membranes were then washed at 65°C in a solution consisting of 0.15 M NaCl/15 mM sodium citrate and 0.1% sodium lauryl sulfate and exposed to Kodak XAR5 x-ray film to obtain autoradiograms. Hybridizing clones were rescreened at low density and isolated. Plasmid DNA was isolated from these positive clones and was digested with restriction enzymes and mapped. Further analysis was performed by using Southern blot hybridization. After subcloning appropriate DNA restriction fragments into pGEM-3 plasmid vectors, DNA sequencing was accomplished by using the dideoxy chain-terminating method (19). The SP6 and T7 promoter primers, dATP[α-³⁵S], Klenow fragment of *E. coli* DNA polymerase or avian myeloblastosis virus reverse transcriptase were all used in the performance of sequencing (20). Sequences of two cDNA clones were obtained in their entirety. DNA sequence was determined by overlapping across all restriction enzyme sites used as initiation points.

Placental poly(A)⁺ RNA was prepared as described (14) and was separated by electrophoresis through 1% agarose/formaldehyde denaturing minigels (21). After electrophoresis the RNA was transferred to nitrocellulose membranes by capillary blotting in the presence of a 3 M NaCl/0.3 M sodium citrate, pH 7.0, solution. Nitrocellulose mem-

Abbreviations: IGF, insulin-like growth factor; UTR, untranslated region.

§To whom reprint requests should be addressed.

¶This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg), (accession no. J03242).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

branes were baked in a vacuum oven for 2 hr at 80°C. *Hind*III restriction fragments of λ DNA and the RNA ladder were concomitantly electrophoresed in lanes adjacent to the poly(A)⁺ RNA, and lanes containing specific-size bands were cut from the gel and stained with ethidium bromide for sizing. Hybridizations were performed by using [α -³²P]dCTP-labeled, nick-translated IGF-II cDNA probes (1×10^6 cpm/ml) in a solution of 35% (vol/vol) formamide, 0.9 M NaCl/90 mM sodium citrate, 0.1 M sodium phosphate (pH 7.2), Denhardt's solution, 20 μ g of sonicated/denatured *E. coli* DNA per ml, 10% dextran sulfate, and 0.1% sodium lauryl sulfate at 42°C. Hybridized blots were washed in a solution of 0.015 M NaCl/1.5 mM sodium citrate at 65°C before exposure to Kodak XAR5 x-ray film.

RESULTS AND DISCUSSION

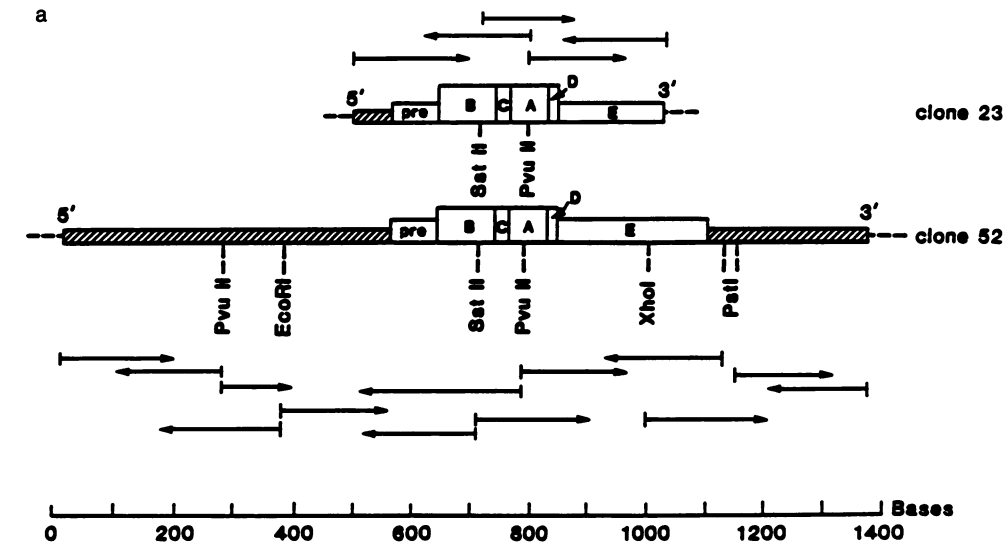
By using an adult human liver IGF-II cDNA insert containing the coding region (14) as probe, cDNAs encoding a human IGF-II sequence were isolated from a placental library. This library was prepared by using poly(A)⁺ RNA obtained from a term human placenta of an overt diabetic. Because IGF-II RNA appears to be present in greater relative abundance in overt diabetic placenta as compared with that in normal pregnancy placenta (14), an unamplified overt diabetic placental cDNA library was used for screening. Approximately 30,000 colonies from this cDNA library were screened, of which 23 individual colonies specifically hybridized to the human liver IGF-II probe. Analysis of the cDNA inserts of these clones revealed the presence of two groups differing in size. One clone from each group was selected for further examination. Clone 23 contained a cDNA insert of \approx 550 base pairs (bp) and clone 52 contained an insert of \approx 1350 bp. These inserts were analyzed by restriction mapping and DNA sequencing. Fig. 1*a* illustrates the restriction maps of IGF-II clone 23 and clone 52 cDNA inserts and depicts the strategy used for DNA sequencing. Clone 23 contains part of the base sequence for the 5'-UTR and a portion of the protein coding region. The entire sequence of the clone 23 cDNA insert is present in clone 52. The DNA sequence in the overlapping region of these clones is identical. The sequence of the 1350-bp insert of clone 52 (Fig. 1*b*) contains an open reading frame of 540 bp, from which the sequence of a 180-amino acid protein can be predicted. This protein contains the sequence for the signal peptide, the B, C, A, and D domains of mature human IGF-II, and includes the entire E domain (17). The predicted polypeptide has 94% amino acid sequence identity with rat IGF-II (16). The 5'-UTR of clone 52 is composed of 546 bases and the 3'-UTR of this clone contains 264 bases. Comparison with the human IGF-II genomic sequence (16, 22) indicates that the 5'-UTR and the 3'-UTR are incomplete. A possible reason for this is high G+C content (>65%) in the 5'-UTR and a similar structural barrier in the 3'-UTR of the placental IGF-II mRNA. By comparing the sequence of clone 52 with IGF-II cDNA sequences isolated from adult liver cDNA libraries (23, 24), it is apparent that the coding regions and the 3'-UTRs are identical, whereas the 5'-UTRs are completely different, except for 8 bases upstream from the initiation codon, ATG (Fig. 1*b*; ATG is underlined). Examination of published human IGF-II genomic sequences (11, 15, 22) indicated two potential RNA splice sites, which, if utilized, would result in formation of two human IGF-II mRNAs differing in their 5'-UTRs. One of these has been identified in IGF-II cDNA from adult liver and the other is represented in clone 52 from placenta. Both species are most probably derived from a single IGF-II gene by way of differing intron-exon organization in the 5'-UTR (Fig. 1*c*). The IGF-II cDNA sequence obtained from an adult human liver library matches the exon gene sequences of

IGF-IIA (11, 22) in which the 5'-UTR is interrupted by at least three introns of 11, 1.5, and >6 kb in length (Fig. 1*c*). The IGF-II cDNA sequence obtained from the placental library matches the exon gene sequences of human IGF-IIB (16) in which the 5'-UTR is interrupted by at least one intron of 2.68 kb (Fig. 1*c*). Since only one IGF-II gene has been detected in the human genome (11), the 5' exon (exon -1) of IGF-IIB may be contained within the large intron (11 kb) that separates exon 1 from exon -1 of IGF-IIA.

RNA molecules transcribed from a single gene but containing heterogeneous 5'-UTRs have been reported for a few eukaryotic genes. These transcripts may arise by different mechanisms. One such mechanism is single promoter usage with alternative transcription initiation and/or alternative splicing (25). Another possible mechanism is alternative promoter usage (26-29). Frunzio *et al.* (29) reported that the IGF-II gene of rat BRL-3A cells contains two distinct promoter regions that are transcribed with differing efficiency, resulting in the production of RNAs having different 5' noncoding exons. However, since the 5'-UTR sequences of the IGF-II cDNA derived from the human adult liver and placenta are not complete, the mechanism(s) responsible for the heterogeneous 5'-UTRs of these two human IGF-II mRNAs is, at present, unresolved and needs to be established. It has been suggested that the human IGF-II gene also contains two distinct promoters (17).

The nucleotide sequences of the IGF-II cDNA from placenta are identical to the suggested exons -1, 1, 2, and 3 of the IGF-IIB gene (Fig. 1*c*) reported by Dull *et al.* (16). Therefore, the suggested exon-intron assignments for this gene are confirmed. Only a minor difference exists in the IGF-II cDNA from placenta. This difference occurs in the base sequence from positions -168 to -159 where a stretch of 10 deoxyadenosine residues is present (Fig. 1*b*; underlined). In contrast, the IGF-IIB gene contains a stretch of only 8 deoxyadenosine residues in the same relative sequence positions. Since sequencing of the IGF-II cDNA from placenta has been performed twice in this particular area, using two different subcloned fragments, and autoradiograms clearly showed 10 distinct adenosine bands, sequencing errors are unlikely to be the reason for the discrepancy. This difference may be the result of a reverse transcription error that occurred during cDNA synthesis, although this kind of error is rare. However, if our cDNA sequence from positions -159 to -168 is correct, there is an in-frame termination codon (TAA), at position -444 upstream from the ATG initiation codon (Fig. 1*b*; boxed) instead of an in-frame TGA termination codon at position -279, as suggested by the genomic DNA sequence (16). However, in both cases the methionine at position -24 is the first amino acid in the prepro-IGF-II molecule since no other ATG is in phase between Met-24 and the TAA termination codon. Furthermore, comparison of sequences of the human placental IGF-II cDNA to those of buffalo rat liver IGF-II cDNA (16) indicates 80% identity in the amino acid sequences of the coding regions. However, no apparent base sequence identity exists in the 3'-UTRs. In contrast the 5'-UTRs have 85% identity, overall, in their base sequences, with many areas having >90% homology (highly conserved areas) and few areas having <70% identity (variable areas) (Fig. 2). This high degree of sequence conservation in the IGF-II 5'-UTRs may imply possible evolutionary and/or functional significance.

We recently reported that adult human liver-derived IGF-II cDNA, consisting of the 5'-UTR and protein coding region specifically hybridized to four (6.0, 4.9, 3.2, and 2.2 kb) placental poly(A)⁺ RNAs (14). These IGF-II transcripts were present at each stage of gestation and the 6.0-kb RNA was always the most abundant relative to the other three RNAs. Since the 5'-UTR of the IGF-II cDNA isolated from



b

```

5'-TTCTCCCGCAACCTTCCTTCGTCTCCCTCCGGTCCCGCCAGCTCTAGCTCTCGACTCCCTCCCGCCCTCAGGCCCGCCCTCTGGCTTCGGGAACCA -453
AAGTGGATTAATTACAGGCTTTCTGTTCTCTCGCTGTCTCTCTCCCGCTGTGGCCGCTGGCCGCTCTGGCTCTCCCTCTCCCGCTCCCTCTCTT -353
CGCCCCCCCCTTTTCAAGTTCACCTCTCTCTCTCCAGCTATCTCTGCCGCCCTCTATCCTTGATACACACAGCTGACCTCAATTTCCCGATACCTTTTCCCCC -253
CGAAAAGTACAAACATCTGGCCCGCCAGCCGGAAGACAGCCGCTCTCCCTGGACAATCAGAGGAATTCGCCCGCCCCCCCAGAAAAAAGCCATC -153
CCCCGGCTCTGCCCGGTGCACATTCGGCCCGGGACTGGCCGAGAGGGGGCTGGCAGAGGAGTCTCGGGCAGGAGGCCAACGCCCGCTGTGGCTT -53
                    -24                -20
                    Met Gly Ile Pro Met Gly Lys Ser Met Leu Val Leu
                    ATG GCA ATC CCA ATG GGG AAG TCG ATG CTG CTG CTT 36

-10                1                10
Leu Thr Phe Leu Ala Phe Ala Ser Cys Cys Ile Ala Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu
CTC ACC TTC TTG GCC TTC GCC TCG TGC TGC ATT GCT GCT TAC CGC CCC AGT GAG ACC CTG TGC GCC GGG GAG CTG 111

                20                30
Val Asp Thr Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyn Phe Ser Arg Pro Ala Ser Arg Val Ser Arg Arg
GTG GAC ACC CTC CAG TTC GTC TGT GGG GAC CGC GGC TTC TAC TTC AGC AGC CCC GCA AGC OGT GTG AGC OGT CGC 186

40                50                60
Ser Arg Gly Ile Val Glu Glu Cys Cys Phe Arg Ser Cys Asp Leu Ala Leu Leu Clu Thr Tyr Cys Ala Thr Pro
ACC OGT GGC ATC GTT GAG TCC TGT TTC GGC AGC TGT GAC CTG GCC CTC CTG CAG AGG TAC TGT GCT ACC CCC 261

70                80
Ala Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg Tyr Pro Val Gly Lys
GCC AAG TCC GAG AGG GAC GTC TGC ACC CCT CGC ACC GTG CTT CGC GAC AAC TTC CCC AGA TAC CCC CTC GGC AAG 336

90                100                110
Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg
TTC TTC CAA TAT GAC ACC TGG AAG CAG TCC ACC CAG GGC CTT GGC AGG GGC CTG OCT GCC CTC CTG OCT GCC CGC 411

120                130
Arg Gly His Val Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His Arg Pro Leu Ile Ala Leu Pro
CGG GGT CAC GTC CTC CCC AAG CAG CTC CAG GGC TTC AGG GAG GCC AAA GGT CAC GGT CCC CTC AIT GCT CTA CCC 486

140                150                156
Thr Gln Asp Pro Ala His Gly Gly Ala Pro Pro Glu Met Ala Ser Asn Arg Lys ***
ACC CAA GAC CCC GCC CAC GGG GCC CCC CCA GAG ATG GCC AGC AAT OGG AAG TGA GCAAAACTGCCGCAAGTCTGCAG 566
CCCCGGCCACCATCTCTCAGCCTCTCTCTCAACCAAGGAAGTTTCCATCAGGTTCCATCCCGAAATCTCTGGTTCCAGTCCCGCTGGGGCTTCTCTCT 666
GACCCAGTCCCGCTGCCCGCCCTCCCGAAGAGGCTACTCTCTCTCGGCCCTCCATCGGGCTGAGGAAGCACAGCCAGCATTTCAAAATCTACAAAA 766
TGCATTCGGCTTTAAACACCCCTTCACTACCCCTCCCGCC-3' 804
    
```

c

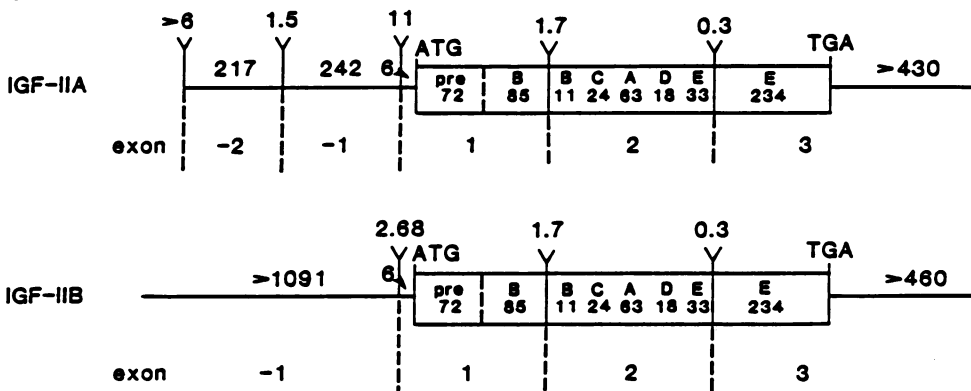


FIG. 1. (a) Organization of and sequencing strategy used for the two placental cDNAs encoding human IGF-II. The regions encoding the B, C, A, and D domains (taller open boxes) and those coding for the signal and E peptides (shorter open boxes) are depicted. The 5'- and 3'-UTRs are presented as hatched rectangles. Only the restriction enzyme sites used in sequencing are indicated. The horizontal arrows show the direction and extent of the sequence determinations. (b) Nucleotide sequence of placental cDNA encoding IGF-II. Nucleotides are numbered sequentially from the initiation codon, ATG, which codes for the methionine residue at amino acid position -24, and nucleotides preceding this ATG codon are given negative numbers. The predicted amino acid sequence is numbered beginning with the amino terminus of the IGF-II sequence (underlined). Amino acid residues comprising the signal peptide are given negative numbers. (c) Organization of IGF-II gene. The IGF-IIA and IGF-IIB gene organizations depicted are from de Pagter-Holthuisen *et al.* (22) and Dull *et al.* (16), respectively. The sizes of the exons and the coding domains are presented in bases. Intron positions are identified by the symbol Y, and intron sizes are presented in kb.

```

human TTCTCCGGCAACCTTCCCTTGCTGCTGCC TCCGCTGCC C TCTAGCTCCGACTCCCTCCGGGCTTCCGGCTTGGCGG 95
rat GC-TA-C-T-AG-C-T-C-T-C-TT-C-A-T-----T-----

human AACCAAAGTGGATTAAATTAGACGCTTTCTGTTTCTCTCGGTGCTGTTCTCTCCGGCTGTCGGCTCCGGCTTCCGCTTGGCTGCTCCTCTCTCCGGCTGCGCT 195
rat T-C-T-----T-----C-----A-----T-----T-----

human CTCCTTGG CCCCCCTTTTCAGGTTCACTGCTGCTCTCCGCACTATCTCTGCCGGCTT CTATCTGTGATAGACAGCTGACCTCATTTCGGATACCTT 293
rat GT-T-----T-----AA-----

human TTCGGCCCC GAAAAGTACACATCTGCCGGGCCCCGAGCCGAAGAGAGCCGCTCTCCCTGGGAACTCAGAGAAATTTCCGGGCCCCGGGGCAAAAA 391
rat CC-----GT-----T-----T-----AA-G-G-A-GA GG-----T-----TC-----

human AAAGCCTATCCCGGCTCTG CCGCGTGGCAGATTGGCCCCGGGAGACTGGCCAGAGGGGGCTGGCAGAGGAGTCTCCGGCAGGAGGGGCAAGCCGG 490
rat T-----T-----T-----T-----A-----C-----A-----TT-----

human CTCCTTGGTGGTGG ACACGGCAGCGGGAGGTGGGGCGGAGGCTGGCGGCTTCCAGACACCA-3' 552
rat AT-C-----A-----GT-----
    
```

FIG. 2. Comparison of the 5'-UTR sequences of human placental and rat liver IGF-II cDNA. The sequence of rat liver IGF-II cDNA was determined by Dull et al. (16) from the rat liver cell line BRL-3A. Nucleotide differences in the sequences are indicated and nucleotide identity is represented by a dash. Spaces indicate the absence of nucleotides in one sequence as compared to the other sequence.

the human placental library differs from that of the IGF-II cDNA isolated from an adult human liver library, it was of interest to determine whether the 5'-UTR of the adult liver IGF-II cDNA was present in one or more of the four major IGF-II transcripts detected in the human placental poly(A)⁺ RNA population. Therefore, the 5'-UTRs of placental IGF-II cDNA and adult liver IGF-II cDNA were used as probes of human placental poly(A)⁺ RNA. A 266-bp placenta-derived IGF-II cDNA probe was prepared that consists of the sequence beginning 281 bases upstream from the initiation codon, ATG (Fig. 3, probe A), and contains a portion of the 5'-UTR of placental IGF-II cDNA. Another probe of 123 bp was prepared from adult liver-derived IGF-II cDNA (23) consisting of the sequence beginning 190 bases upstream from the initiation codon, ATG (Fig. 3, probe B), and probe containing a portion of the 5'-UTR of the adult liver IGF-II cDNA. These cDNA fragments were used to probe poly(A)⁺ RNA from human placentas of each gestational stage in RNA transfer blot hybridizations. Probe A specifically hybridized to three of the four previously detected placental IGF-II transcripts. The sizes of the hybridizing IGF-II RNAs were 6.0, 3.2, and 2.2 kb (Fig. 3a). These three transcripts were present at each stage of gestation. Term placenta from gestational and overt diabetic pregnancies seemingly express relatively more of these three IGF-II RNAs than term placenta from normal pregnancy. These data are in agreement with our previous observations that indicated that the expression of IGF-II RNA was increased in term placenta of overt and gestational diabetic pregnan-

cies relative to normal term placenta (14). This previous determination was made by using the adult human liver IGF-II cDNA, consisting of the 5'-UTR and protein coding region, as probe. In contrast, when probe B was used in hybridization experiments with placental poly(A)⁺ RNA as target, no transcripts were detected. As a control, adult human liver-derived IGF-II cDNA, which consists of 5'-UTR and protein coding region (probe C), was used to probe placental poly(A)⁺ RNA. As expected, probe C specifically hybridized to four IGF-II RNAs of 6.0, 4.9, 3.2, and 2.2 kb in size (Fig. 3c). Therefore, analysis of the results of these hybridizations using probes A, B, and C lead to the conclusion that the four IGF-II transcripts detected in human placenta most probably contain the same base sequence in their coding regions as that represented in the adult human liver IGF-II cDNA protein coding region. Further, it is apparent that these four placental IGF-II transcripts probably do not contain the same base sequence in their 5'-UTRs as that in the 5'-UTR of adult human liver IGF-II cDNA. The 6.0-, 3.2-, and 2.2-kb IGF-II transcripts detected in placental poly(A)⁺ RNA appear to contain the same base sequence in their 5'-UTRs as that in the 5'-UTR of the IGF-II cDNA isolated from the placental library. The 4.9-kb placental IGF-II transcript apparently does not contain this 5'-UTR. Thus, the equivalent of the 5.3-kb IGF-II RNA expressed in the adult human liver (11) does not appear to be present in the IGF-II transcript population of human placenta. This transcript may be in extremely low abundance and its presence in placenta requires clarification.

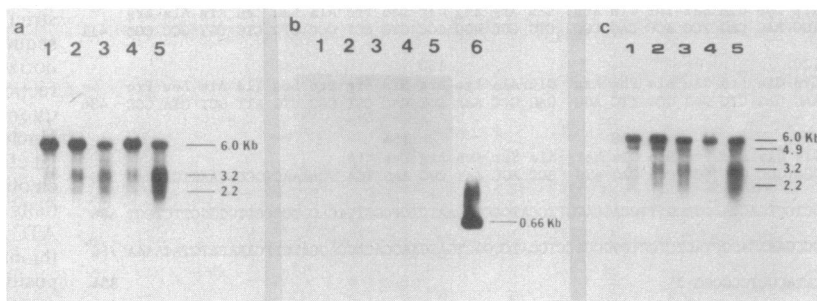


FIG. 3. Hybridization of IGF-II cDNA probes to blots of placental poly(A)⁺ RNA from differing stages of gestation. Gestational stages of the placental poly(A)⁺ RNA are first trimester/11–12 weeks (lanes 1), second trimester/29 weeks (lanes 2), normal term (lanes 3), term of a gestational diabetic pregnancy (lanes 4), and term of an overt diabetic (class B) pregnancy (lanes 5). For the blot shown in a, the 5'-UTR of placental IGF-II cDNA was used as probe (designated probe A). This fragment consists of 266 bp beginning 281 bases upstream from the coding region (*Pvu II*/*Nae I* fragment of placental IGF-II cDNA). Probe A was nick-translated to a specific activity of 1 × 10⁷ cpm/μg of DNA, and an autoradiogram from hybridization of the blot in a was obtained by exposure to x-ray film for 2 days with two intensifying screens at -70°C. For the blot shown in b, the 5'-UTR of adult liver IGF-II cDNA was used as probe (15) (designated probe B). This fragment consists of 123 bp beginning 190 bases upstream from the coding region. Probe B was nick-translated to a specific activity of 6 × 10⁷ cpm/μg of DNA, and an autoradiogram was obtained as above. Lane 6 of the blot in b contains 100 ng of the adult liver IGF-II cDNA insert of 660 bp from which probe B was obtained. This insert is composed of coding region flanked by 5'-UTR, which is 190 bp. It was hybridized with probe B as positive control. An autoradiogram from hybridization of the blot shown in b, lane 6 was obtained by exposure to XAR5 x-ray film for 2 hr with two intensifying screens at -70°C. For the blot shown in c, adult liver IGF-II cDNA insert (660 bp) was used as probe (probe C). This insert consists of 5'-UTR (190 bp) and coding region (15). Probe C was nick-translated to a specific activity of 2 × 10⁷ cpm/μg of DNA, and an autoradiogram of the blot in c was obtained as described above for RNA blots. Lanes 1–5 in the blots shown in a and b contain 2 μg each of placental poly(A)⁺ RNA. Lanes 1 and 3–5 in the blot shown in c contain 2.5 μg each of placental poly(A)⁺ RNA and lane 2 contains 3.0 μg. The sizes of the hybridizing transcripts are presented in kb. *HindIII* fragments of either λ DNA or RNA ladder (Bethesda Research Laboratories) were used to size.

In review, the 6.0-, 3.2-, and 2.2-kb IGF-II RNAs of human placenta appear to contain the same base sequence as those determined in clone 52. In contrast, the 4.9-kb IGF-II RNA of human placenta appears to contain the same base sequence as that determined for the protein coding region in clone 52. This transcript, however, does not contain a 5'-UTR base sequence homologous to that in either the 5'-UTR of clone 52 or the 5'-UTR of adult liver cDNA, suggesting the presence of a third base sequence in the 5'-UTR of human IGF-II RNA. The functional significance of these different IGF-II RNA 5'-UTRs is not known. In comparison, adult human kidney expresses two IGF-II transcripts of 6.0 and 4.9 kb. The 4.9-kb transcript appears to be present in greater relative abundance than the 6.0-kb transcript (11). This 4.9-kb IGF-II transcript is similar in size to the placental IGF-II transcript that apparently contains a third unique, as yet unsequenced, 5'-UTR, while maintaining the presence of the same coding region present in placental and liver IGF-II transcripts.

Heterogenous 5'-UTRs of IGF-II RNAs transcribed from a single gene may serve a regulatory function. The existence of these different IGF-II transcripts is representative of tissue-specific, IGF-II gene expression, as their presence varies in placenta, liver, and possibly kidney. Such tissue-specific expression has been demonstrated for mouse amylase (30, 31), the *Drosophila Adh* gene (28), and the myosin light chains (32). These IGF-II transcripts, differing in their 5'-UTRs, may also be representative of alternative developmental gene expression. Some placental IGF-II transcripts may represent fetal IGF-II gene expression, whereas adult liver IGF-II RNA may only represent adult IGF-II gene expression. Recently, by using a genomic probe it was shown that human fetal liver may express IGF-II transcripts that are different from adult liver transcripts at the 5'-UTR (17). However, human fetal liver IGF-II sequences have not been cloned. Moreover, since many fetal tissues express IGF-II, the same analysis should be applied to them before concluding that it is a general phenomenon of different expression regarding fetal and adult tissues. It is possible that placental IGF-II expression as compared to adult liver represents alternative developmental expression. Such developmental switching of expression has been demonstrated for the *Drosophila Adh* gene (28) and the myosin light chains (32). The specific function(s) of the differing IGF-II RNA 5'-UTRs is yet to be elucidated.

Finally, it should be noted that the BRL-3A rat liver cell line, expressing IGF-II RNA containing a 5'-UTR sequence with extensive identity to our sequence (16), was derived from adult rat liver. Following culture these cells exhibited a fetal pattern of RNA expression (33). This might indicate that the IGF-II RNA containing human placental-homologous IGF-II 5'-UTR is a developmentally regulated transcript expressed in fetal tissues and some tumors (17). The conserved IGF-II RNA 5'-UTRs may, therefore, be regulatory in function.

While this manuscript was being handled for publication, data supportive of that stated here were reported. LeBouc *et al.* (34) sequenced an IGF-II cDNA clone from human placenta with an identical, but considerably shorter, 5'-UTR, as that reported here. Irminger *et al.* (35) also reported an identical, but considerably shorter, IGF-II 5'-UTR from an adult hypothalamic cDNA clone. Lastly, Gray *et al.* (36), using the rat BRL-3A 5'-UTR with homology to that reported here, showed tissue-specific and developmental expression of human IGF-II RNAs possessing this sequence.

We thank Ms. Joyce Olson for preparation of this manuscript. This work has been supported by National Institutes of Health Grant HD-18271, National Research Service Award F-32-HD06782 to S.-J.S. from the National Institutes of Child Health and Human Development, National Institutes of Health Developmental Biology Training Grant HD-010704, and the American Diabetes Association.

- Rinderknecht, E. & Humbel, R. E. (1978) *J. Biol. Chem.* **253**, 2769-2776.
- Rinderknecht, E. & Humbel, R. E. (1978) *FEBS Lett.* **89**, 283-289.
- Froesch, E. R., Zapf, J. & Humbel, R. E. (1983) in *Diabetes Mellitus*, eds. Ellenburg, M. & Riskin, M. (Medical Examination, New Hyde Park, NY), 3rd Ed., pp. 179-201.
- Clemmons, D. R. & Van Wyk, J. J. (1981) in *Tissue Growth Factors*, ed. Baserga, R. (Springer, Berlin), pp. 161-208.
- Van Wyk, J. J. (1984) in *Human Proteins and Peptides*, ed. Li, C. H. (Academic, New York), Vol. 12, pp. 81-125.
- Zapf, J., Schmid, C. & Froesch, E. B. (1984) in *Clinics in Endocrinology and Metabolism: Tissue Growth Factors*, ed. Daughaday, W. H. (Saunders, London), Vol. 13, pp. 3-30.
- Sara, V. R., Hall, K., Misaki, M., Frykland, L., Christensen, N. & Wetterberg, L. (1983) *J. Clin. Invest.* **71**, 1084-1094.
- Weidman, E. R. & Bala, R. M. (1980) *Biochem. Biophys. Res. Commun.* **92**, 577-585.
- D'Ercole, A. J., Applewhite, G. T. & Underwood, L. E. (1980) *Dev. Biol.* **75**, 315-328.
- Adams, S. O., Nissely, S. P., Handwerger, S. & Rechler, M. M. (1983) *Nature (London)* **302**, 150-153.
- Bell, G. I., Gerhard, D. S., Fong, N. M., Sanchez-Pescador, R. & Rall, L. B. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6450-6454.
- Reeve, A. E., Eccles, M. R., Wilkins, R. J., Bell, G. I. & Millow, L. J. (1985) *Nature (London)* **317**, 258-260.
- Scott, J., Cowell, J., Robertson, M. E., Priestley, L. M., Wade, R., Hopkins, B., Pritchard, J., Bell, G. I., Rall, L. B., Graham, C. F. & Knott, T. J. (1985) *Nature (London)* **317**, 260-262.
- Shen, S.-J., Wang, C.-Y., Nelson, K. K., Jansen, M. & Ilan, J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9179-9182.
- Jansen, M., Van Schaik, F. M. A., Ricker, A. T., Bullock, B., Woods, D. E., Gabbay, K. H., Nussbaum, A. L., Sussenbach, J. S. & Van den Brande, J. L. (1983) *Nature (London)* **306**, 609-611.
- Dull, T. J., Gray, A., Hayflick, J. S. & Ullrich, A. (1984) *Nature (London)* **310**, 777-781.
- de Pagter-Holthuisen, P., Jansen, M., van Schaik, F. M. A., van der Kammen, R., Oostervrij, K., Van den Brande, J. L. & Sussenbach, J. S. (1987) *FEBS Lett.* **214**, 259-264.
- Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263-269.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
- Williams, S. A., Slatko, B. E., Moran, L. S. & Desimone, S. M. (1986) *Bio Techniques* **4**, 138-147.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 202-203.
- de Pagter-Holthuisen, P., van Schaik, F. M. A., Verduijn, G. M., van Ommen, G. J. B., Bouma, B. N., Jansen, M. & Sussenbach, J. S. (1986) *FEBS Lett.* **195**, 179-184.
- Jansen, M., van Schaik, F. M. A., van Tol, H., Van den Brande, J. L. & Sussenbach, J. S. (1985) *FEBS Lett.* **179**, 243-246.
- Bell, G. I., Merryweather, J. P., Sanchez-Pescador, R., Stempien, M. M., Priestley, L., Scott, J. & Rall, L. B. (1984) *Nature (London)* **310**, 775-777.
- Reynolds, G. A., Goldstein, J. L. & Brown, M. S. (1985) *J. Biol. Chem.* **260**, 10369-10377.
- Batley, J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, G. & Leder, P. (1983) *Cell* **34**, 779-787.
- Carlson, M. & Botstein, D. (1982) *Cell* **28**, 145-154.
- Benyajati, C., Spoerel, N., Haymerle, H. & Ashburner, M. (1983) *Cell* **33**, 125-133.
- Frunzio, R., Chiariotti, L., Brown, A. L., Graham, D. E., Rechler, M. M. & Bruni, C. B. (1986) *J. Biol. Chem.* **261**, 17138-17149.
- Young, R. A., Hagenbuchle, O. & Schibler, U. (1981) *Cell* **23**, 451-458.
- Schibler, U., Hagenbuchle, O., Wellauer, P. K. & Pittet, A. C. (1983) *Cell* **35**, 501-508.
- Periasamy, M., Strehler, E. E., Garfinkel, L. I., Gubits, R. M., Ruiz-Opazo, N. & Nadal-Ginard, B. (1984) *J. Biol. Chem.* **259**, 13595-13604.
- Soares, M. B., Turken, A., Ishii, D., Mills, L., Episkopov, V., Cotter, S., Zeithin, S. & Efstratiadis, A. (1986) *J. Mol. Biol.* **192**, 737-752.
- LeBouc, Y., Noguez, P., Sondermeijer, P., Dreyer, D., Girard, F. & Binoux, M. (1987) *FEBS Lett.* **222**, 181-185.
- Irminger, J.-C., Rosen, K. M., Humbel, R. E. & Villa-Komaroff, L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6330-6334.
- Gray, A., Tam, A. W., Dull, T. J., Hayflick, J., Pintar, J., Cavenee, W. K., Koufos, A. & Ullrich, A. (1987) *DNA* **6**, 283-295.