Developmental regulation of bovine insulin-like growth factor-II (IGF-II) gene expression: homology between bovine transcripts and human IGF-II exons

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ABSTRACT

Initial observations have indicated similarities between bovine and human IGF-II production during development. The aim of the present study was to investigate whether cattle could provide an experimental model that would mimic the complex pattern of human IGF-II gene expression. Expression of bovine IGF-II gene during development was studied by RNA hybridization using various human IGF-II probes. In fetal tissues and in adult muscle, the bovine IGF-II gene was expressed as a family of eight transcripts ranging in size from 5.2 to 1.1 kb. In adult bovine liver, a major IGF-II transcript of 4.4 kb was expressed that could not be detected in any fetal or adult extra-hepatic tissue. During fetal life, quantitative IGF-II mRNA expression differed in liver and muscle, and the relative amounts of the different transcripts varied with the tissue of origin. These observations suggest that the regulation of bovine IGF-II gene expression is specific to the stage of development and the tissue concerned. Moreover its pattern is very similar to that in its human counterpart.

In order to identify a putative homology between human and bovine gene structures, bovine mRNAs were examined for cross-hybridization with various non-coding exons of the human gene. Cross-hybridization was detected with human untranslated exons 5 and 6, suggesting the presence of two distinct promoters similar to the human promoters P3 and P4. The 4.4 kb mRNA species expressed in adult bovine liver failed to hybridize to a probe for human exons 1 and 2, suggesting that the leader sequences of this transcript were different from those present in the human gene. Finally, results obtained with a probe containing the 3' untranslated end of exon 9 suggested the presence of at least two polyadenylation sites in the bovine gene.

Although differences in IGF-II gene structures were found between cattle and man, the similarities in the pattern of gene expression between the two species suggest that cattle may be a useful model to investigate some developmental aspects of the expression of the human IGF-II gene.

Journal of Molecular Endocrinology (1993) 11, 117–128

INTRODUCTION

Insulin-like growth factor-I (IGF-I) and IGF-II are structurally related peptides that can stimulate the proliferation and differentiation of a variety of cell types (reviewed in Humbel, 1990). Although the function of IGF-II is not fully characterized, it has been suggested to play a central role in the regulation of tissue differentiation and fetal growth. IGF-II transcripts have been identified early in gestation in a wide variety of fetal tissues (Lund et al. 1986; Gray et al. 1987; Han et al. 1987). Accordingly, high levels of circulating IGF-II have been detected in the fetal serum of numerous species (Moses et al. 1980; Gluckman & Butler, 1983; Ashton et al. 1985; Lassarre et al. 1991). Recently, the importance of IGF-II in stimulating fetal growth was clearly demonstrated by De Chiara et al. (1990), who observed marked fetal growth retardation in mice bearing a selectively disrupted
IGF-II allele. These observations suggest both endocrine and paracrine/autocrine modes of action for IGF-II.

The structural organization of the human and rodent IGF-II genes has been extensively described (Ueno et al. 1988; Holthuizen et al. 1990). In man and rat, as well as in other species, the IGF-II gene is transcribed to produce multiple mature mRNAs of different sizes (reviewed in Sussenbach, 1989). Structural analysis of the human and rodent IGF-II genes has revealed multiple 5' untranslated leader exons under the control of specific promoters, as well as alternative polyadenylation signals.

The regulation of IGF-II gene expression is complex and exhibits species-specificity. In man, the liver has been shown to have a tissue-specific pattern of expression during development, with the generation of a 5.3 kb transcript in adult liver. This transcript is absent in fetal liver and in extra-hepatic adult tissues and arises from a specific promoter, PI (de Pagter-Holthuizen et al. 1987). In rat and mouse, however, the IGF-II gene is no longer expressed in the liver after birth and no liver-specific promoter homologous to human promoter PI has so far been detected in the genes of either species.

We have been searching for an experimental model that would mimic the complex pattern of IGF-II expression observed in man. Strong homologies have been shown to exist between the peptide sequences of human and bovine IGF-IIs, as well as between the coding regions of their genes (Honneger & Humbel, 1986; Brown et al. 1990). More interestingly, serum concentrations of bovine IGF-II are higher in adults than in the fetus (Coxam, 1990), which is also observed in man but not in most of the mammalian species so far studied (Daughaday & Rotwein, 1989; Mesiano et al. 1989). Together, these observations suggest that the bovine model may be of interest. We therefore examined IGF-II gene expression in bovine liver through the fetal, neonatal and adult periods to determine whether, as in man, the bovine IGF-II gene exhibits a specific pattern of expression in this tissue. Skeletal muscle was chosen as a reference tissue because, at least in man and rats, there is no modification of promoter usage during development in this tissue (Tollefsen et al. 1989). Possible analogies between the human and bovine gene structures were also analysed using human IGF-II probes containing various untranslated exons.

Although differences in IGF-II gene structure were found between cattle and man, we show that the pattern of bovine IGF-II gene expression during development is very similar to that described in man. This suggests that the bovine model may be useful for the investigation of some aspects of human IGF-II gene expression.

**MATERIALS AND METHODS**

**Animals**

This study was carried out as part of a research programme approved by the Institut National de la Recherche Agronomique (INRA) Ethical Committee. Animals were bred and slaughtered and samples collected at the INRA Research Center (Theix, France) according to ethical guidelines related to animal care.

**Tissue samples**

Pregnant Charolais cows were slaughtered and the fetuses removed by Caesarian section. Samples of bovine liver and skeletal muscle (semi-tendinosus muscle) were dissected from the mothers and fetuses, immediately frozen in liquid nitrogen and stored at −80 °C. Since the cows were artificially inseminated, precise fetal ages were known, the oldest fetus being taken at term (280 days in utero). The neonatal samples refer to liver biopsies from 40- and 100-day-old calves (two calves per sample). These were thoroughly washed in a solution of 0.9% saline, then frozen and stored as described above.

**DNA probes**

All the probes used in this study were derived from the human IGF-II gene. Their mapping on the gene is described in Fig. 1.

The IGF-II coding probe consisted of a 663 bp fragment containing the entire coding sequence in addition to 15 bp of untranslated leader exon 5 and 99 bp of 3' untranslated sequence (probe d, Fig. 1) (Le Bouc et al. 1987). The exon 1–2 probe (probe a, Fig. 1) was a cDNA fragment comprising 65 bp of exon 1 and 220 bp of exon 2 (de Pagter-Holthuizen et al. 1987).

The exon 5 and exon 9 probes (probes b and e respectively, Fig. 1) were derived from human genomic DNA. The exon 5 probe contained 854 bp of the internal sequence of the exon (from +150 to +1004, where +1 is the transcription initiation site). The exon 9 probe was a 1012 bp fragment containing distal sequences of the 3' end of the exon (+2501 to +3513).

The 160 bp exon 6 probe (probe c, Fig. 1) comprised the whole exon and 66 bp of 3' intronic sequences. This probe was generated by polymerase chain reaction (Perkin Elmer Cetus, Irvine, CA,
U.S.A.) using the sense and antisense primers (1 μM each in the reaction mixture) 5'-ACAT TAGCTTCTCCTGTGAAAG-3' and 5'-GAGG TCGGACTCCGTCCCGACTGT-3' and human genomic DNA (1 μg) as a template. The DNA probes were labelled with [α-32P]dATP (3000 Ci/mmol; Amersham International plc, Amersham, Bucks, U.K.) by random priming using a kit (Amersham International plc) according to the manufacturer's instructions. The standard specific activity was 1–2 × 10^6 c.p.m./μg DNA.

**DNA isolation and Southern blot analysis**

Isolation of leukocyte DNA and Southern blot analysis were performed as described previously, using AvaII as restriction enzyme (Schneid et al. 1992).

**RNA preparation**

Total RNA was prepared from frozen tissue samples using the guanidinium isothiocyanate method (Chomczynski & Sacchi, 1987). The amount of RNA was measured by u.v. spectrometry and its integrity checked by analytical agarose gel electrophoresis after ethidium bromide staining. Poly(A)^+ RNA was obtained using one cycle of oligo(dT) cellulose chromatography (Aviv & Leder, 1972).

**Northern blot analysis**

RNA samples were denatured and size-fractionated on a 1.2% agarose gel using either formaldehyde (Lehrach et al. 1977) or glyoxal (MacMaster & Carmichael, 1977) as RNA-denaturing agents. They were transferred to nylon membranes (Hybond-N, Amersham International plc, for the formaldehyde method, or Gene screen, NEN, Boston, MA, U.S.A., for the glyoxal method). RNAs were cross-linked to the membrane by baking at 80 °C for 2 h. Prehybridization was performed at 42 °C for 5 h in 50% formamide, 5 × SSPE (0.9 M NaCl, 0.05 M sodium phosphate, 5 mM EDTA, pH 7), 5 × Denhardt’s solution (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone), 0.1% (w/v) sodium dodecyl sulphate (SDS) and 100 μg salmon sperm DNA/ml. Hybridization (42 °C, 24 h) was performed in the same solution, except that salmon sperm DNA was replaced by 5% dextran sulphate.

When IGF-II cDNA was used as a probe, the blots were washed twice in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7), 0.1% SDS for 15 min at room temperature, then once in 0.1 × SSC, 0.1% SDS for 30 min at 65 °C. When the untranscribed exon probes were used, the final wash was performed in 0.1 × SSC, 0.1% SDS for 30 min at 65 °C for the exon 5 probe, for 20 min at 45 °C for the exon 6 probe and for 30 min at 50 °C for the exon 9 probe. The blots were then exposed to X-ray films (Curix-RP1; Agfa Gevaert) at −80 °C with intensifying screens for different periods of time.

When necessary, the Northern blots were stripped in 5 mM Tris, pH 8, 2 mM EDTA, 0.01 × Denhart’s solution (2 h at 80 °C), then autoradiographed to confirm complete stripping.

**Dot blots**

For each sample, 16 μg total RNA were denatured with formaldehyde. Twofold serial dilutions were then applied to nylon membranes (Hybond-N)
IGF-II gene expression in fetal tissues

Total RNA samples from fetal bovine liver and skeletal muscle were analysed by Northern blotting and hybridized with a human IGF-II cDNA coding probe (probe d, Fig. 1). In these tissues, the IGF-II gene was expressed as a family of transcripts (Fig. 2). When RNAs were denatured with formaldehyde, seven transcripts of 5-2, 4-0, 3-4, 2-8, 2-1, 1-6 and 1-1 kb were detected (Fig. 2a). The intensities of hybridization signals revealed differences in the respective amounts of these transcripts. For instance, the 2-8 kb mRNA was abundant in both fetal liver and muscle, whereas the 1-1 kb mRNA was poorly expressed. Larger amounts of the 5-2 and 2-1 kb transcripts were detected in muscle than in liver, whereas the 4-0 kb transcript seemed to be more abundant in liver. The 3-4 kb mRNA was only detected in fetal muscle. When formaldehyde was replaced by glyoxal, the same pattern of expression was obtained, with the exception of the 2-1 kb transcript which appeared to be composed of two mRNAs of 2-3 and 2-0 kb (Fig. 2b). When poly(A)* mRNA of the same tissues was hybridized, all these transcripts, except the 1-6 kb mRNA, were detected (data not shown).
Quantitative analysis of IGF-II gene expression during fetal development

In order to quantify total IGF-II mRNA expression in liver and muscle during fetal development, dot blots were performed using the same cDNA coding probe as above (Fig. 3). In both liver and muscle, the amounts of fetal IGF-II mRNA are expressed relative to the amounts of IGF-II mRNA for the corresponding adult tissue. Figure 3 shows that IGF-II mRNAs were much more abundant in fetal than in adult tissues, by 4- to 22-fold and 7- to 12-fold in muscle and liver respectively. Moreover, the amounts of IGF-II mRNA varied differently in the two tissues through fetal development. In liver, IGF-II gene expression remained constant throughout gestation, whereas in muscle, after a peak value at around 150 days in utero, it decreased progressively with gestational age (Fig. 3). The decrease observed in muscle did not reflect the selective disappearance of any particular transcript, but rather an overall decrease in all mRNA species (data not shown). In liver, IGF-II gene expression was markedly depressed early in postnatal life, as seen in the biopsy sample from 40-day-old calves (Fig. 3, postnatal sample). These analyses (Figs 2 and 3) therefore reveal both tissue-specific and developmental regulation of IGF-II mRNA expression during fetal life.

IGF-II gene expression during postnatal development

Bovine IGF-II gene expression was analysed in adult liver and compared with expression in fetal liver. A major transcript of 4.4 kb was detected in adult liver (Fig. 4). In this tissue, the transcripts observed during fetal life were either barely or not detectable when compared with the 4.4 kb mRNA. This 4.4 kb mRNA was not detected in fetal liver, fetal muscle or adult muscle (Fig. 4), or in fetal kidney, fetal adrenals or adult adrenals (data not shown). Therefore, in bovine liver, all the IGF-II transcripts expressed during fetal life are switched off and a new type of transcript is expressed in the adult tissue. This liver-specific expression is comparable with that in human liver, where a similar switch in IGF-II expression is observed (Sussenbach, 1989). In order to determine at which point in liver development this changeover takes place, Northern blot analyses were performed with total RNA samples prepared from liver biopsies of 40- and 100-day-old calves; these ages correspond to weights which are twice and three times birth weight respectively (Fig. 5). In these postnatal tissues, an intermediate pattern of IGF-II gene

FIGURE 3. Quantitative analysis of total IGF-II mRNAs in bovine (a) muscle and (b) liver during development. Total RNA was prepared from muscle and liver from bovine fetuses (113, 150, 240, 270 or 280 days in utero) and from the livers of 40-day-old calves (postnatal sample). Dot blots were performed as described in the Materials and Methods, and were hybridized with the human coding IGF-II probe (probe d, Fig. 1). Autoradiograms of dot blots were analysed by densitometry and the values obtained for fetal and postnatal tissues were expressed relative to the values for the corresponding adult tissue which was used as a standard. Each point represents the mean ± S.D. of three different dot blots where different RNA preparations from the same tissue were loaded; ND, not determined.
expression was obtained, with the presence of the 5.2, 2.8 and 2.0 kb IGF-II transcripts, already observed in fetal liver, and an additional transcript of 4.2–4.3 kb. Whether this 4.2–4.3 kb transcript is identical to the liver-specific 4.4 kb transcript has not yet been determined. The amounts of IGF-II mRNA in the liver had, however, decreased dramatically after birth, as was seen with dot blot analysis (Fig. 2).

Analysis of homologies between bovine IGF-II transcripts and human IGF-II untranslated exons

The developmental pattern of IGF-II gene expression in bovine tissues proved to be very similar to that observed in man. This prompted us to search for sequence similarities and structural homology between the bovine and human genes, using specific probes for several parts of the human IGF-II gene (Fig. 1). As mentioned in the Materials and Methods, the stringency of the final wash differed from probe to probe, and this should reflect the degree of sequence similarity found between the two species for each untranslated sequence. Hybridization specificity was tested using total RNA from human hepatocarcinoma or adult human liver as internal controls. In addition, after hybridization with the various probes, the Northern blots were stripped and rehybridized with the IGF-II cDNA coding probe.

When Northern blots were hybridized with a probe containing exon 5 (probe b, Fig. 1), four bovine IGF-II transcripts (5.2, 3.4, 2.8 and 2.1 kb) were detected in both fetal liver and skeletal muscle (Fig. 6a, lanes 3 and 4). In contrast, the probe containing exon 6 (probe c, Fig. 1) hybridized with the bovine fetal IGF-II mRNAs of 4.0 and 1.1 kb (the 1.1 kb transcript being barely detected in different experiments) (Fig. 6b, lanes 3 and 4). A smear was obtained with the exon 6 probe in the presence of adult liver poly(A)⁺ mRNA (Fig. 6b, lane 2), probably due to the weak stringency of the final wash of the membrane. As previously described (de Pagter-Holthuizen et al. 1987, 1988), probes b (exon 5) and c (exon 6) hybridized to human hepatocarcinoma controls with the human IGF-II mRNAs of 6.0 and 2.2 kb and 4.8 kb respectively (Fig. 6a and b, lane 5). Neither probe hybridized with the liver-specific bovine 4.4 kb transcript (Fig. 6a and b, lanes 1 and 2).

In order to determine whether, as in man, several polyadenylation signals occur on the bovine IGF-II gene, a probe for exon 9 was tested, which extended into the 3' untranslated end of the exon (probe c, Fig. 1). In bovine tissues, this probe revealed the mRNAs of 5.2, 4.4, 4.0 and 2.1 kb, as well as an additional 1.4 kb transcript, which was not detected using the IGF-II cDNA coding probe (Fig. 7a and b, lanes 1, 2 and 3). In the control (human hepatocarcinoma), this probe recognized the human IGF-II mRNAs ending at the distal polyadenylation site (mRNAs of 6.0 and 4.8 kb) and the 1.8 kb transcript (Fig. 7a, lane 4) (de Pagter-Holthuizen et al. 1988).

As in man, adult bovine liver expresses a specific transcript. It therefore was of interest to hybridize bovine mRNAs from adult liver with a probe containing the non-coding exons 1 and 2 (probe a, Fig. 1), which are present in the human liver-specific IGF-II transcript of 5.3 kb (Sussebnach, 1989). Northern blots hybridized with this probe failed to give any signal in any of the bovine tissues.
examined (either fetal or adult). However, this probe specifically recognized the 5.3 kb mRNA expressed in adult human liver used as control (data not shown). By way of further confirmation of these results, the possibility that the untranslated exons 1 and 2 exist in bovine genomic DNA was investigated by Southern blot analysis using the same probe (probe a). As shown in Fig. 8a, no restriction fragments were detected in bovine genomic DNA with the probe for exons 1 and 2, whereas the presence of these exons was confirmed in the human genomic DNA. As expected, the control IGF-II cDNA coding probe (probe d, Fig. 1) detected restriction fragments in both bovine and human genomic DNA (Fig. 8b).

**DISCUSSION**

The aim of our study was to investigate a suitable animal model that would mimic the pattern of IGF-II gene expression observed in man, particularly in the liver. We therefore examined bovine IGF-II gene expression in fetal, neonatal and adult liver, using skeletal muscle as an extra-hepatic control.
In both fetal bovine tissues and in adult bovine muscle, multiple mRNA species were detected, similar to the pattern observed in other mammals (Lund et al. 1986; Irminger et al. 1987; Hedley et al. 1989; O'Mahoney et al. 1991). In man, rat and mouse, these multiple mRNAs arise from the presence of several leader exons preceded by various promoters and from multiple polyadenylation sites at the 3' end of the gene (Sussenbach, 1989). In the case of the human IGF-II gene, four different promoters (P1-P4) have been identified (Holthuizen et al. 1990).

Using specific probes encompassing different untranslated exons of the human IGF-II gene, we have been able to identify the sequences which are contained in these various bovine IGF-II mRNAs. Among the seven IGF-II transcripts expressed in fetal and extra-hepatic adult bovine tissues, four cross-hybridized with human exon 5 (probe b; mRNAs of 5-2, 3-4, 2-8 and 2-1 kb) and two with human exon 6 (probe c; mRNAs of 4-0 and 1-1 kb) (Fig. 6). These results indicate strong sequence homologies between bovine and human leader exons 5 and 6, and suggest the existence of bovine promoters homologous to human promoters P3 and P4.

Results obtained with a probe for human exon 9 (probe e) suggest that the bovine IGF-II gene might contain multiple polyadenylation signals. This probe, encoding the 3' untranslated end of exon 9, is located close to the more distal polyadenylation site of the human IGF-II gene (de Pagter-Holthuizen et al. 1988). This probe recognized four bovine IGF-II mRNAs of 4-4 kb (derived from adult liver), 5-2 kb and 2-1 kb (containing exon 5) and 4-0 kb (containing exon 6). Apart from the result for the bovine 2-1 kb transcript, these findings are similar to those for man, where the same probe (probe e) recognizes the liver-specific 5-3 kb mRNA and mRNAs containing exon 5 (6-0 kb) and exon 6 (4-8 kb), ending at the more distal polyadenylation site. It is therefore possible that, as in man, the bovine 5-2, 4-4 and 4-0 kb transcripts contain the same polyadenylation site. The bovine 3-4 and 2-8 kb transcripts detected with the exon 5-specific probe or the 1-1 kb mRNA detected with the exon 6 probe failed to hybridize with the 3' end of human exon 9. Since these bovine transcripts were shorter, this could mean that they end at a more proximal polyadenylation site on the bovine IGF-II gene. In this respect, hybridization of the 2-1 kb transcript with the 3' end of exon 9 is surprising in view of its size. However, other mechanisms, such as alternative splicing or degradation of a longer mRNA, cannot be excluded. The 1-4 kb transcript detected in bovine tissues (with
probe e only) strongly suggests homology with the 1.8 kb mRNA detected in human tissues with the same probe (de Pagter-Holthuizen et al. 1988). As has been suggested for the human 1.8 kb mRNA, the bovine 1.4 kb mRNA may be of physiological relevance, regulating IGF-II mRNA levels through endonucleolytic cleavage (Meinsma et al. 1991).

Bovine IGF-II gene expression exhibited slight tissue-related differences. In skeletal muscle, three major IGF-II transcripts (5.2, 2.8 and 2.1 kb) arose from the same promoter (cross-hybridizing with human exon 5), whereas in fetal liver the two major transcripts (2.8 kb and 4.0 kb) may be generated from two distinct promoters (cross-hybridizing with human exons 5 and 6 respectively). This suggests that, as occurs in human tissues, promoter usage might differ from one tissue to another (Irminger et al. 1987). The relative amounts of the transcripts were also different in liver and muscle, and it seems possible that differences exist in the translatability and/or stability of the various bovine mRNAs, as has been suggested for rats and man (Graham et al. 1986; Sussenbach et al. 1991). Quantitatively, the developmental profiles of total IGF-II mRNAs through gestation were different in the two bovine tissues studied. Levels remained constant in the liver, but decreased with gestational age in muscle. A similar stability in the amounts of IGF-II mRNAs has been described in ovine fetal liver and seems to be specific for this tissue (O'Mahoney et al. 1991). The markedly smaller amounts of IGF-II mRNA in adult than in fetal bovine liver are also the same as has been reported for other species (Lund et al. 1986; Irminger et al. 1987; Hedley et al. 1989; O'Mahoney et al. 1991).

Bovine liver IGF-II gene expression exhibited developmental stage- and tissue-specific regulation. The major 4.4 kb transcript expressed in adult liver was not detected in any other fetal or adult tissue. The same developmental modification of hepatic IGF-II gene expression has been described in man, pigs and sheep, but not in rats (Lund et al. 1986; de Pagter-Holthuizen et al. 1987; Hedley et al. 1989; O'Mahoney et al. 1991). In man, the expression of a specific IGF-II transcript in adult liver corresponds to the activation of a particular promoter, P1, which
is inactive in fetal or extra-hepatic adult tissues (Sussenbach, 1989). Transcription from promoter P1 includes the leader exon 1 and the untranslated exons 2 and 3 at the 5' end of the liver-specific 5.3 kb transcript. Although it seems likely that the 4.4 kb transcript expressed in adult liver is the bovine counterpart of the human 5.3 kb transcript, it did not cross-hybridize with the probe for human exons 1 and 2. This was confirmed by Southern blot analysis, showing these sequences to be absent in bovine genomic DNA. These findings are in agreement with earlier studies showing that the 5' end of the human IGF-II gene (promoter P1 and exons 1, 2 and 3) is poorly conserved among species. This region is indeed absent in rats and mice (Lund et al. 1986; Rotwein & Hall, 1990). In pigs and sheep, liver-specific IGF-II cDNAs from adult liver libraries have been cloned. In these two species, the first 230 bp of 5' untranslated sequences closest to the initiation codon are homologous to human exon 3 (74 and 64% homologies in pigs and sheep respectively) (Catchpole & Engström, 1990; O'Mahoney et al. 1991). Sequences further upstream are known only for porcine cDNA where, compared with human genomic DNA, there is 75% homology with human exon 1 but no homology at all with human exon 2 (Catchpole & Engström, 1990). However, in pigs and sheep, the sequence of the promoter controlling IGF-II gene expression in the adult liver is not yet known.

The timing of the developmental switch in promoters that occurs in human liver remains unknown. Our preliminary studies show that in bovine liver only slight changes occur in the pattern of IGF-II gene expression up to 3 months after birth. This would suggest that acquisition of the liver-specific pattern of IGF-II expression is not induced by delivery, but arises progressively during postnatal life.

In conclusion, we present here a description of IGF-II gene expression in bovine liver during development, and an initial characterization of the IGF-II bovine gene structure compared with the human gene structure. We have shown that bovine IGF-II gene expression during development shares strong similarities with human IGF-II gene expression, particularly with the appearance of a specific IGF-II transcript in adult liver. However, differences exist in the IGF-II gene structures of the two species. Northern blot and Southern blot analyses have failed to detect any similarities between cattle and man in the 5' untranslated fragments of the adult liver-specific transcripts. This does not exclude the possibility that critical regulatory sequences are conserved in the bovine IGF-II gene. Sequence analysis of the bovine liver-specific 4.4 kb transcript and binding assays of bovine liver nuclear extracts to the human P1 promoter will be useful in analysing whether the cow is definitively a suitable model for the study of molecular mechanisms regulating human IGF-II gene expression. Nevertheless, our data on IGF-II gene expression during postnatal life show that the bovine model can be used to investigate the evolution of IGF-II gene expression in the liver after birth, and more particularly the timing of the switch in the IGF-II mRNA profile which is specific to this tissue.

ACKNOWLEDGEMENTS

We thank D. Seurin for her technical assistance.

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Institut National de la Recherche
Agronomique (AIP no. 90/4714), a collaborative grant from INSERM and the Netherlands Organization for Advancement of Pure Research (NWO), the Association de Recherche Contre le Cancer, and the Ligue Contre le Cancer. H. Schneid was a recipient of a fellowship of the Fondation pour la Recherche Medicale.

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RECEIVED 6 January 1993