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Gene expression of the insulin-like growth factor system during mouse kidney development

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Abstract

Expression of the insulin-like growth factor (IGF) system was investigated in mouse renal development and physiology, using non radioactive in situ hybridization and quantitative RT-PCR. IGF-I mRNA levels increased after birth and were confined to distal tubules and peritubular capillaries in the outer medulla. IGF-II mRNA levels were high in developing kidneys and peaked after birth. The type I receptor mRNA expression pattern mostly parallelled those of IGF-I and IGF-II. The IGF binding proteins (IGFBP's) showed weak mRNA expression for IGFBP-1 and -6. High fetal mRNA levels were measured for IGFBP-2, showing a similar profile in time as observed for IGF-II. Low fetal IGFBP-3 and -5 mRNA levels increased after birth. IGFBP-2, - 4 and -5 mRNA expression was localized to differentiating cells. In the mature kidney predominant expression was confined to proximal tubules (IGFBP-4), thin limbs of Henle's Loop (IGFBP-2), glomerular mesangial cells (IGFBP-5) and peritubular capillaries of the medulla (IGFBP-5). IGFBP-3 mRNA was exclusively expressed in endothelial cells of the renal capillary system. Distinct mRNA expression for each member of the IGF system may point to specific roles in development and physiology of the mouse kidney. © 1997 Elsevier Science Ireland Ltd.

Keywords: Development; Mouse kidney; IGF; IGFBPs; mRNA expression

1. Introduction

The development of the kidney is based on morphogenic processes that evolve in a sequential manner. After an inductive interaction of the epithelial cells of the ureter and the mesenchymal cells of the metanephros, both cell types start to proliferate and differentiate, finally leading to the formation of glomeruli and renal tubules (Saxen and Sariola, 1987).

A number of polypeptide growth factors are involved in renal organogenesis. Epidermal growth factor (EGF), transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs) and insulin-like growth factors (IGFs) have been described in developmental studies for their regulatory roles in morphogenesis (Hammerman, 1995). Both IGF-I and IGF-II are single chain polypeptides which have mitogenic and differentiating effects on many cell types. IGF action is predominantly mediated through the type I receptor and the availability of the IGFs for the receptor is thought to be regulated by the IGF binding proteins (IGFBPs).

The kidney is an obvious target of IGFs. In addition to circulating IGFs, local synthesis implies paracrine and autocrine IGF action as well. In the mature kidney IGFs can alter proximal tubular transport processes (Mellas et al., 1986; Rogers and Hammerman, 1989; Quigly and Baum, 1991; Caverzasio et al., 1990), stimu-

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late vectorial Na^+ transport (Blazer-Yost and Cox, 1988; Blazer-Yost et al., 1989) and control glomerular filtration rate and renal plasma flow levels (Hirschberg et al., 1991, 1993).

IGF-I and IGF-II have been detected in rat metanephroi (Rogers et al., 1991) and abundant IGF-II expression was found in epithelial and mesenchymal cells of the nephrogenic zones in man (Matsell et al., 1994). IGF-I expression was more prominent after development, mainly confined to the thick ascending limbs of Henle's Loop in both rat and man (Chin et al., 1992; Rabkin et al., 1995). The type I IGF receptor is expressed in the rat mesonephros during early embryogenesis (Bondy et al., 1990) and blocked expression in mouse kidney organ cultures (Liu et al., 1993) has demonstrated its relevance in nephrogenesis. After development the type I receptor is widely distributed in kidney of rat (Chin et al., 1992; Rabkin et al., 1995) and man (Chin et al., 1994; Chin and Bondy, 1992).

Six mouse IGFBPs have been cloned (Schuller et al., 1994), each of them showing specific expression patterns during embryonic development (Schuller et al., 1993). During development of the human kidney abundant expression is seen for IGFBP-2 and -4 in nephrogenic zones and epithelial cells of the maturing glomerulus, while moderate expression is seen for IGFBP-3 in epithelial cells of the ureteric bud and for IGFBP-5 in originating mesenchymal cells (Matsell et al., 1994). In the mature rat kidney glomerular and tubular expression is described for IGFBP-2 and -4, where as IGFBP- 3 and -5 were mainly interstitially expressed (Rabkin et al., 1995; Price et al., 1995). Low expression of IGFBP-1 is found in the adult human kidney (Chin et al., 1994), while it has been well detected in the thick ascending limbs of Henle's Loop in rat (Chin et al., 1992; Rabkin et al., 1995; Price et al., 1995). The discrete, spatial and temporal expression patterns of the IGFBP genes in kidney support their modulatory role in IGF action.

To study kidney pathophysiology mouse models are often used. We therefore aimed in this study to examine the involvement of the IGF system in normal mouse kidney development. A non radioactive in situ hybridization technique was performed in order to precisely localize the mRNAs of the IGF system in mouse fetal and postnatal kidneys. Total mRNA content for IGF-I, IGF-II, IGFBP-2,-3 and - 5 in fetal and early postnatal kidneys was determined by quantitative RT-PCR. Combining both techniques resulted in a detailed description of the gene expression of the IGF system during mouse kidney development.

2. Materials and methods

The experimental procedures reported in this study were carried out in accordance with the Dutch Animal and Experimentation Act after approval was granted by the Ethical Committee on Animal Experimentation of the Erasmus University Rotterdam.

2.1. Tissue isolation

Kidneys were collected from BALB/c mice on fetal day E13, 14, 15, 17, 19 and postnatal day P0, 1, 3, 7, 14. They were either stored in $-80°C$ for total RNA extraction or fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin according to standard procedures. For in situ hybridization, 4 μm sections were cut on a microtome and mounted on 3-aminopropyl trioxysilane-coated slides. Sections were dried at 37°C for 3 days.

2.2. Probe preparation

The IGFBP-2 to -6 cRNA probes were transcribed from templates described by (Schuller et al., 1994) . As template for the IGFBP-1 cRNA probe the mouse cDNA fragment Sph1-Sac1 was cloned into pTZ18R or pTZ19R (Pharmacia, Uppsala, Sweden) for the antisense or sense probes, respectively. cDNAs encoding mouse IGF-I and -II were kindly provided by Dr G.I. Bell (Howard Hughes Medical Institute, Chicago, IL). Fragments were subcloned into pTZ18 and pTZ19 (EcoR1 for IGF-I and BamH1/Sac1 for IGF-II). A 265 bp EcoR1/Sma1 fragment of the rat type I IGF receptor cDNA clone ligated in pGEM3 (Promega, Madison, WI) was kindly provided by Dr H. Werner and Dr D. LeRoith (National Institutes of Health, Bethesda, MD). Digoxigenin-11-UTP labeled RNA probes were prepared according to the manufacturer's prescription (Boehringer Mannheim GmbH, Biochemica, Mannheim, Germany) using T7 or SP6 RNA polymerase.

2.3. In situ hybridization

Sections were dewaxed, hydrated and incubated in the following solutions: 0.2 N HCL, 0.3% Triton-X 100 in PBS, 5 μg/ml Proteinase K (37°C), 4% formalin in PBS and finally acetylated with acetic anhydride diluted in 0.1 M triethanolamine (750 μl/200 ml). Until hybridization sections were stored in a solution of 50% formamide in 2 × SSC at 37°C. For hybridization, probes were diluted in hybridization solution (50% deionized formamide, 10% dextran sulfate, 2 × SSC, 1 × Denhardt's solution, 1 μg/ml tRNA, 250 μg/ml herring sperm DNA) to a concentration of 100 ng/ml, incubated at 68°C for 15 min and layered onto the

sections. Sections were hybridized overnight at 55°C in a humid chamber. Posthybridization washes were performed at 45°C using the following steps: 50% formamide in 2 × SSC, 50% formamide in 1 × SSC and 0.1 × SSC. A 15 min incubation with RNase T1 (2 U/ml in 1 mM EDTA in 2 × SSC) at 37°C was followed by washes of 0.1 × SSC at 45°C and 2 × SSC at room temperature. The DIG-labeled hybrids were detected by antibody incubation performed according to the manufacturer's prescription (Boehringer Mannheim GmbH, Biochemica, Germany) with following modifications. A 1:2000 dilution of anti-digoxigenin (Fab) conjugated to alkaline phosphatase was used for a 2.5 h incubation at room temperature. Afterwards an extra washing step of 0.025% Tween in Tris buffered saline pH 7.5 was introduced. For staining, sections were layered with detection buffer (0.1 M Tris–HCl, 0.1 M NaCl, 0.05 M MgCl$_2$ pH 9.5) containing 0.33 mg/ml NBT (4-nitroblue tetrazolium chloride), 0.16 mg/ml BCIP (5-bromo-4-chloro-3-indolyl-phosphate), 7.5% PVA (polyvinylalcohol, m.w. 31 000–50 000 Aldrich Chemical Milwaukee, WI) and 1 mM levamisol (Sigma, St. Louis, MO). The colour reaction was performed in the dark and was stopped when the desired intensity of the resulting blue precipitate was reached. Sections were washed in 10 mM Tris–HCl, 1 mM EDTA pH 8.0, counterstained with PAS and Nuclear red solution, dehydrated with ethanol gradients and mounted with an ethanol based mounting medium Euparal (Chroma-Gesellschaft, Stuttgart, Germany). Control sections for morphological analysis were stained with hematoxylin and eosin.

2.4. RNA extraction

RNA preparation was carried out according to the acidic phenol/chloroform method of Chomczynski and Sacchi (1987) from frozen samples conserved at −80°C. Each time point was represented by a pool of corresponding mouse kidneys. The recovery of total RNA was measured by spectrophotometric analysis at 260 nm and quality and quantity were verified on a 1.5% agarose gel. Similar intensities were observed in all samples.

2.5. Quantitative RT-PCR

Quantitation of mRNA was done by the non-competitive PCR method described by Pane et al. (1995). An absolute amount of mRNA molecules is calculated by interpolating the generated amount of PCR products from sample RNA into a titration curve obtained by amplifying a known number of synthetic RNA molecules containing the same sequence as the RNA to be quantitated.

2.5.1. Preparation of standard RNA

The synthetic mRNA used as standard was obtained in two steps. First, cDNA fragments containing the desired sequences were amplified by performing PCR on the available cDNAs of the mouse IGF system (Schuller et al., 1993). After purification and densitometric quantitation, the fragments were ligated for 3 h at 15°C into the pGEM-T vector (Promega, Madison, WI). This vector contains the T7 promoter sequence and is specially adapted to ligate PCR fragments. A second PCR was performed using 1 μl ligation mixture (1:1 diluted) as DNA template, the T7 promoter sequence as 5′-primer (5′- TGTAATACGACTCAC-TATAGGGAGA-3′) and gene specific 3′-primers (Table 1). This strategy resulted in a PCR fragment that contained the same sequence as to be quantitated plus the T7 promoter sequence. This T7-fragment was purified and used for in vitro RNA transcription (Ribo-MAX kit, Promega, Madison, WI). The produced cRNA was purified by phenol-chloroform extraction and Sephadex G50 column elution. The number of molecules was calculated by using the molecular weight of the cRNA molecule, Avogadro's number and the spectrophotometric absorbance at 260 nm.

2.5.2. RT-PCR

RT-PCR was performed on both prepared standard RNA and extracted sample RNA One microgram total RNA was reverse transcribed with 200 units of M-MLV reverse transcriptase (Gibco BRL) primed with specific 3′-primers for IGF-I, IGF-

Table 1
Sequences of PCR primers and predicted PCR products

Amplified mRNAs	Sense and antisense primers	Size of amplified products (bp)
IGF-I	5′-AAAATCAGCAGTCT-TCCAAC-3′	
	5′-AGATCACAG CTCCG-GAAGCA-3′	299
IGF-II	5′-GCCCCGGAGAGACT-CTGTGCG-3′	
	5′-GCCCACGGGGTATC-TGGGGAA-3′	253
IGFBP-2	5′-TGGAGGAGCCCAA-GAAGCT-3′	
	5′-GGTTCACACACCAG-CAACTC-3′	228
IGFBP-3	5′-GCCGCGGGCTCT-GCGTCAACGC-3′	
	5′-CTGGGACTCAGCA-CATTGAGGAAC-3′	415
IGFBP-5	5′-TTGCCTCAACGAAA-AGAGC-3′	
	5′-AGAATCCTTTGCG-GTCACA-3′	377

II, IGFBP-2, -3 and -5 (Table 1) in a final volume of 20 μl. The PCR reaction was carried out in a final volume of 50 μl with the equivalent of 50 ng of total RNA (5 μl of a 1:5 dilution of the Reverse Transcription reaction) in PCR buffer (50 mM KCl, 1.5 mM MgCl$_2$, and 20 mM Tris–HCl, pH 8.3) plus 200 μM deoxy-NTPs, [α-P^{32}]dATP, 25 pmol forward and reverse primers and 1 U of Hi-Taq DNA Polymerase (Bioprobe Systems, France). PCR reactions were performed separately using an initial denaturation at 94°C for 3 min, followed by 25 cycles that included: 30 s at 94°C, 20 s annealing at 53°C (except for IGFBP-2, 45°C) and 30 s at 72°C. Products were analysed on a 2.5% agarose gel and bands corresponding to each specific PCR product were excised and the amount of incorporated radioactivity was determined by β-scintillation counting. Oligonucleotide primers designed for the amplification were purchased from Pharmacia Biotech (The Netherlands).

2.6. Statistical analysis

The differences in expression levels during development were scored for significance using the one-way analysis of variance (ANOVA), followed by a Scheffe F test. Level of significance was chosen at $P < 0.05$.

3. Results

Development of the mouse kidney starts at fetal day E10 when the ureteric bud emerges and invades the mesenchyme. The resulting appositional development of renal vesicles continues until the early postnatal period. All stages can be recognized in sections of mouse fetal day E15 (staging performed according to Saxen (1987)) and were therefore used to present the expression patterns in developing structures (Fig. 1A,1B). The mRNA expression patterns in mature structures (Fig. 1C) are represented by data from kidneys at postnatal day P14. Scoring based on results from the in situ hybridization of E15 and P14 are listed in Tables 2 and 3, respectively. Results from the RT-PCR quantitation are presented in Fig. 5.

3.1. In situ hybridization

3.1.1. IGF-I mRNA

A scattered pattern of IGF-I mRNA expression in all cell types was observed during kidney development (Fig. 2A). The expression pattern at birth was confined to the epithelial cells of both layers of Bowman's capsule and the epithelial cells of cortical tubules. After day P7 a rather drastic decrease was seen in the proximal tubules and at day P14 most of the hybridization signal was found in the peritubular capillaries of the outer medulla and inner cortex (Fig. 4A).
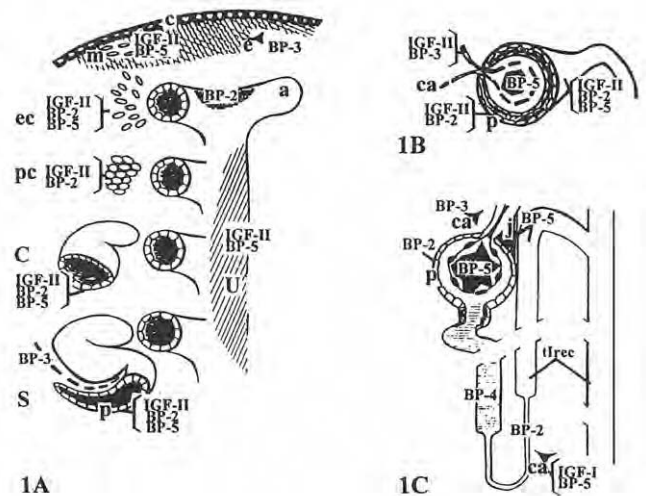

Fig. 1. Schematic illustration of the main expression sites of the IGF system in the developing mouse kidney. (1A) Developmental stages during nephrogenesis including: early condensate (ec); pretubular condensate (pc); comma-shaped body (C); and S-shaped nephron (S). (1B) Developing glomerulus. (1C) Mature nephron. Abbreviations are: c, capsule; m, mesenchyme; e, endothelial cells; a, ampulla; U, ureteric bud; p, parietal epithelial layer; ca, capillary; j, juxtaglomerular cells.

3.1.2. IGF-II mRNA

At different fetal stages strong hybridization signals for IGF-II mRNA were detected in differentiating mesenchymal and epithelial cells, as well as endothelial cells. No signals were detected in epithelial cells of the ampulla of the ureteric bud (Fig. 2B). Prominent expression sites were the ureteric bud, parietal and visceral epithelial layers of the nephric bodies (Fig. 2B) and developing proximal tubules (Fig. 4B). After development most cells showed reduction of IGF-II mRNA expression, except for endothelial cells of renal vessels. Faint signals were present in epithelial cells of Bowman's capsule.

3.1.3. Type I receptor mRNA

The type I receptor mRNA was expressed in all cells of the kidney during fetal development (Fig. 2C). Continuous expression from fetal stage until maturity was observed in visceral and parietal epithelial layers of developing glomeruli and in epithelial cells of distal and collecting tubules (Fig. 4C). Type I receptor mRNA expression was found in proximal tubules until birth and until day P7 the thick limbs of Henle's Loop (Fig. 4C) showed expression. Faint hybridization signals were observed at day P14.

3.1.4. IGFBP-1 mRNA

Little or no expression was seen in fetal kidney for IGFBP-1 (Fig. 2D). At day P14 faint signals could be observed in some cells of the proximal tubules, distal tubules and the epithelial cells of Bowman's capsule, while strong signals were seen in control liver sections (data not shown).

Table 2
Expression of IGF system in structures of developing mouse kidney at day E15

	IGFI	IGF II	tIrec	BP1	BP2	BP3	BP4	BP5	BP6
Renal capsule	−	+	+	+	−	−	+	+ +	+
Mesenchymal cells	+	+	+	−	+	+	+	+	+
Early condensate	+	+ +	+	−	+	−	−	+ +	−
Pretubular condensate	+	+ +	+	−	+	−	+	−	−
Endothelial cells	+	+	+	−	+	+ +	+	+	+
Ureteric bud	+	+	+	−	+	−	−	+ +	−
Ampulla	+	−	+	−	+	−	−	−	−
Branching area	+	−	+	−	+ +	−	−	−	−
Comma- and S-shaped bodies and developing glomeruli									
Invading endothelial cells	+	+	+	−	−	+ +	+	−	−
Mesangial cells	+	−	+	−	+	−	+	+ +	−
Visceral epithelial layer	+	+	+	−	−	−	−	−	−
Parietal epithelial layer	+	+ +	+	−	+ +	−	+	+	−
Developing tubules	+	+	+	−	+	−	+	+ +	−

Scoring:— no signal above background; + low abundance; + + high abundance. Scoring is based on observations in a minimum of three kidney sections obtained from at least two animals.

3.1.5. IGFBP-2 mRNA

Early stages of developing kidney showed abundant hybridization signals for IGFBP-2 mRNA in the ureteric bud and the surrounding mesenchyme. At day E15 hybridization signals were strong in the branching area of the ureteric bud (Fig. 2E and Fig. 4D) and the parietal epithelial layer of the developing nephric bodies (Fig. 3A). During maturation expression in the glomerulus was expanded to the visceral epithelial layer and at day P14 both layers of Bowman's capsule contributed to the IGFBP-2 mRNA expression pattern. In contrast to a postnatal decrease of hybridization signals in developing proximal tubules, an increase could be observed in the thin limbs of Henle's Loop, being very strong at day P14 (Fig. 4E).

3.1.6. IGFBP-3 mRNA

Single cells within the population of mesenchymal cells expressed IGFBP-3 mRNA from day E13 on (Fig. 2F). Endothelial cells of the invading capillary loop showed strong expression in the S-shaped nephron stage (Fig. 3B) and during maturation of the glomerulus (Fig. 3D,3F). However in the mature glomerulus no IGFBP-3 mRNA was detected anymore (Fig. 3H). IGFBP- 3 mRNA expression sites at day P14 were the peritubular capillaries in the cortex (Fig. 3H) and to a lesser extent those in the medulla.

3.1.7. IGFBP-4 mRNA

The IGFBP-4 gene was expressed in mesenchymal cells of the developing kidney, showing more signal in the pretubular condensate and in cells enclosed in the nephric bodies and developing glomeruli. No expression was seen in epithelial cells of the ureteric bud and the comma-and S-shaped bodies, while epithelial cells of more developed tubules did express IGFBP-4 mRNA (Fig. 2G). At day P14 the main sites of expression were the proximal tubules including their straight segments (Fig. 4F) and peritubular capillaries in the outer medulla.

3.1.8. IGFBP-5 mRNA

The epithelial cell layer of the renal capsule and the underlaying outer zone of the metanephric blastema expressed IGFBP-5 mRNA throughout nephrogenesis (Fig. 2H). A very marked expression was observed in mesenchymal cells forming the early condensate (Fig. 2H) and those destined to become mesangial cells in developing glomeruli (Fig. 3C). Hybridization signals for IGFBP-5 mRNA were found in epithelial cells of developing proximal tubules (Fig. 4H) and of the ureteric bud, except its ampulla (Fig. 2H). The expression pattern changed as the kidney matured, showing a decrease in proximal tubules from day P7 on and a very strong expression arising in peritubular capillaries of the medulla (Fig. 4G). IGFBP-5 mRNA could also be detected in peritubular capillaries and distal tubules of the cortex and abundant expression was present in mesangial cells and in juxtaglomerular cells of the glomeruli (Fig. 3E,3G).

3.1.9. IGFBP-6 mRNA

IGFBP-6 mRNA was very weakly expressed during nephrogenic stages in epithelial cells (Fig. 2I), but after birth expression could be observed in the visceral epithelial layer and podocytes of developing glomeruli and collecting tubules. At day P14 no hybridization signals were seen. As positive controls, liver sections showed good hybridization signals.

Table 3
Expression of IGF system in mouse kidney at day P14

	IGF I	IGF II	tIrec	BP1	BP2	BP3	BP4	BP5	BP6
Glomeruli									
Capillaries	−	−	−	−	−	−	−	−	−
Mesangial cells	−	−	−	−	−	−	+	+ +	−
Bowman's capsule	+	+	+	+	+ +	−	−	−	−
Juxtaglomerular cells	−	−	−	−	−	−	−	+ +	−
Peritubular capillaries									
Outer cortex	−	−	−	−	−	+ +	−	+	−
Inner cortex	+	−	−	−	−	−	−	−	−
Outer medulla	+ +	−	−	−	−	+	+	+ +	−
Inner medulla	+	−	−	−	−	+	−	+ +	−
Distal tubules	+	−	+	+	+	−	−	+ +	−
Proximal tubules	−	−	−	+	−	−	+ +	+	−
Thick limb of Henle's Loop	+	−	+	−	−	−	+	−	−
Thin limb of Henle's Loop	−	−	−	−	+ +	−	−	−	−

Scoring: − no signal above background; + low abundance; + + high abundance. Scoring is based on observations in a minimum of three kidney sections obtained from at least two animals

Renal vessels expressed IGFBP-2,-3,-4 and -5 mRNA throughout development (data not shown).

3.2. Quantitative RT-PCR

The amount of IGF-II and IGFBP-2 mRNA molecules was high during renal development and for both the same profile was observed: after a significant rise towards birth a postnatal decline started. Lower fetal levels were measured for IGF-I, IGFBP-3 and -5 mRNA and after birth all three began to increase significantly (Fig. 5).

4. Discussion

This study has shown well defined spatial and temporal mRNA expression profiles of the members of the IGF system during mouse nephrogenesis. Our presentation is to our knowledge a first combination of data from non radioactive in situ hybridization and quantitative RT-PCR, resulting in optimal cellular localization and reliable quantitation.

As non radioactive in situ hybridization was meant for optimal localization, the colour reaction was stopped when discriminate hybridization signals with maximum intensities were reached. The quantitative RT-PCR compensated for possible variations in probe specificity, UTP content or hybridization conditions and resulted in absolute amounts of mRNA molecules measured.

We have observed distinct expression patterns for the IGFs, the type I receptor and the six IGFBPs throughout kidney development. The widespread moderate expression of type I receptor mRNA is compatible with its IGF-I and -II signal transducing role. On the other hand, the specifically localized expression of the IGFBP mRNAs is supportive for their modulatory role in IGF actions. The half life of the IGFs is prolonged by binding to the IGFBPs, which in circulation function as carrier proteins providing a reservoir of IGFs. In addition, locally produced IGFBPs can regulate the availability of IGFs in the vicinity of their receptors and thus modulate their actions (Liu et al., 1994).

The inverse quantitation profiles for IGF-I and IGF-II do suggest their distinct role in different stages of development. High prenatal levels of IGF-II mRNA confined to sites of cellular differentiation support a vital function in nephrogenesis, while increasing levels of IGF-I after birth emphasize its role in postnatal kidney physiology. In the mature kidney localization of IGF-I and type I receptor mRNA in the distal tubules favour the involvement of IGF in transmembrane transport of for instance Na^+ and tubular inorganic phosphate (Pi), as has been suggested by others (Kobayashi et al., 1995; Caverzasio et al., 1990). Their presence in epithelial cells of the glomerulus might indicate an involvement in regulation of glomerular filtration processes (Hirschberg et al., 1991).

Close interactions of the IGFs and their binding proteins in developing and physiological processes are indicated by their overlapping or adjacent expression sites and parallelling quantitation profiles.

The low hybridization signals for IGFBP-1 mRNA that we detected in the mouse kidney correspond with the findings of Chin et al. (Chin et al., 1994; Chin and Bondy, 1992) in human kidneys. On the other hand, IGFBP-1 is well expressed in rat (Rabkin et al., 1995; Chin and Bondy, 1992; Price et al., 1995) and levels were shown to be influenced by long-term diabetes
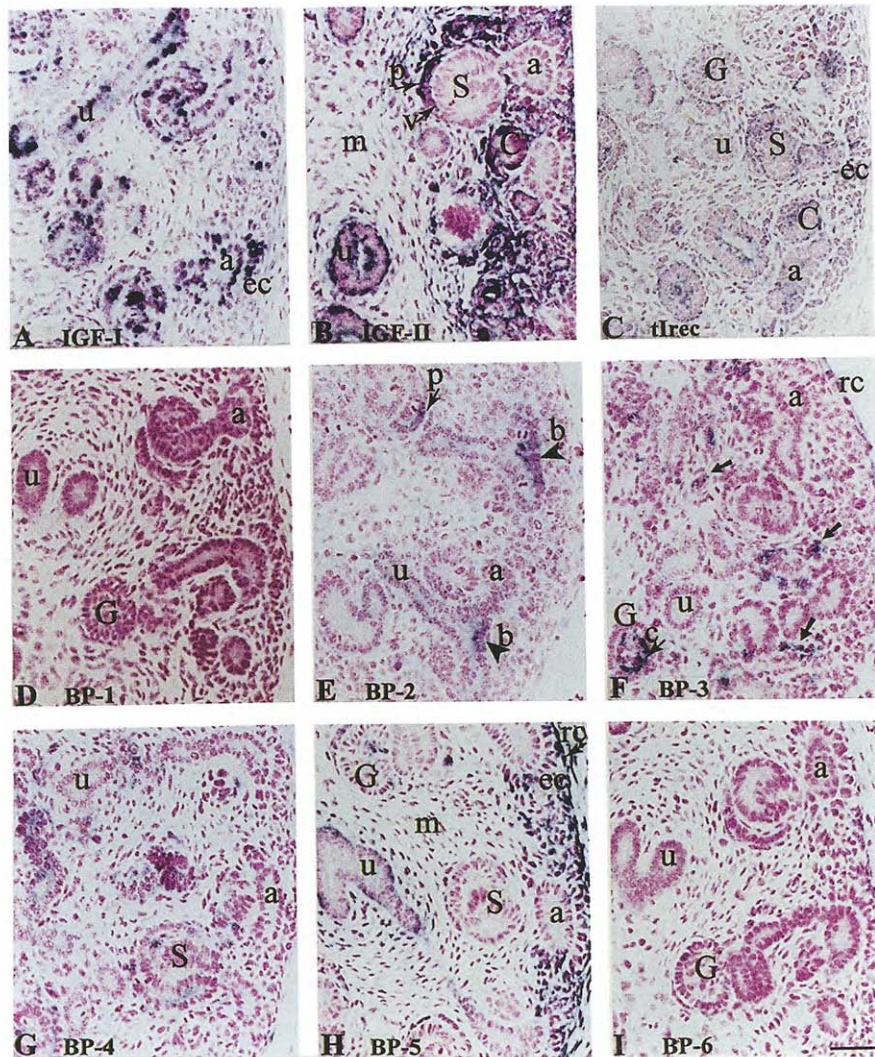
Fetal expression

Fig. 2. Photomicrographs of in situ hybridization with DIG-labeled cRNA probes in developing mouse kidneys at day E15. The blue coloured precipitate corresponds to sites of mRNA expression of (A) IGF-I, (B) IGF-II, (C) type I receptor, (D) IGFBP-1, (E) IGFBP-2, (F) IGFBP-3, (G) IGFBP-4, (H) IGFBP-5, (I) IGFBP-6. Abbreviations are: u, ureteric bud; a, ampulla; m, mesenchyme; ec, early condensate; C, Comma-shaped body; S, S-shaped body; G, glomerulus; p, parietal epithelial cells; v, visceral epithelial cells; b, branching area of the ureteric bud; rc, renal capsule; c, capillary loop. Arrows (↘) indicate expression sites of IGFBP-3 in the early capillary network. Scale bar = 50 μM.

(Landau et al., 1995). These data may indicate a species difference for the role of renal IGFBP-1 in mediating autocrine or paracrine IGF actions.

A striking similarity was observed in the profiles of mRNA levels for IGFBP-2 and IGF-II during nephrogenesis. High prenatal IGFBP-2 mRNA levels which peaked around birth and decreased postnatally, were mimicked by IGF-II. This not only demonstrates the importance of both genes during fetal and perinatal kidney development, but also indicates a close interaction in gene regulation, as has been proposed by others (Delhanty and Han, 1993; Carr et al., 1995). Strong IGFBP-2 hybridization signals were confined to induced differentiating cells in the branching area of the ureteric bud. They support a role for IGFBP-2 in nephrogenic IGF-II actions. Like in rat (Rabkin et al., 1995; Chin and Bondy, 1992; Price et al., 1995) and man (Matsell et al., 1994; Chin et al., 1994; Chin and Bondy, 1992) IGFBP-2 mRNA was confined to the epithelial cells in the mature glomerulus and here its protein might bind circulating or locally produced IGF and facilitate the translocation of vascular IGF into the tubular compartments for renal clearance (Chin and Bondy, 1992). A similar function has been suggested in brain (Bondy et al., 1992). High expression in the thin limbs of Henle's Loop accorded with IGFBP-2 mRNA (Rabkin et al., 1995; Price et al., 1995) and IGFBP-2 protein (Evan et al., 1995) data in rat kidney. By
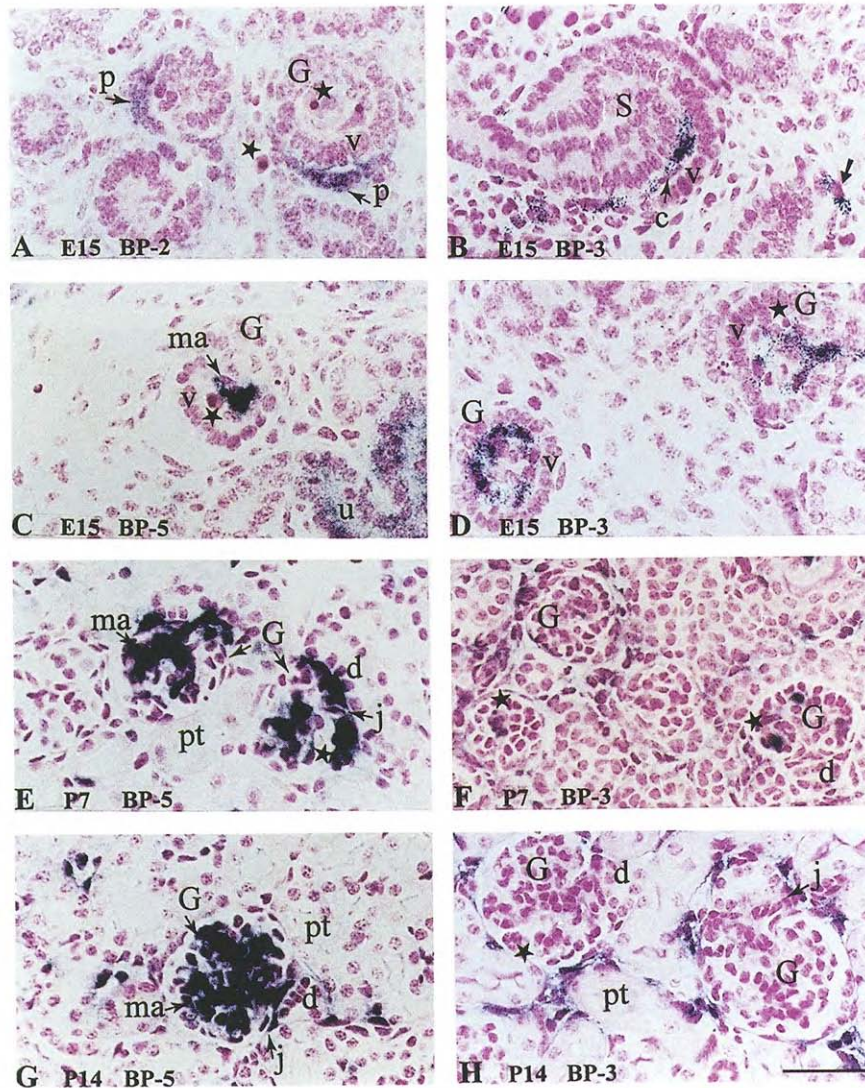
Glomerular expression

Fig. 3. Photomicrographs of in situ hybridization with DIG-labeled cRNA probes in the developing stages of glomeruli in mouse kidney. (A) IGFBP-2 at day E15, (B, D, F, H) IGFBP-3 at day E15, E15, P7 and P14, respectively. (C, E, G) IGFBP-5 at day E15, P7 and P14, respectively. Abbreviations are: u, ureteric bud; S, S-shaped body; G, glomerulus; p, parietal epithelial cells; v, visceral epithelial cells; c, capillary; ma, mesangial cells; pt, proximal tubule; d, distal tubule; j, juxtaglomerular cells; ✱ indicates erythrocyte present in capillary; arrow ⬊ in (B) points to IGFBP-3 expressing endothelial cell in the mesenchyme. Scale bar = 50 μM.

influencing the amount of available IGF-II, IGFBP-2 might modulate IGF-II action in Na^+/H^+ exchange across the membranes of the epithelial cells, as has been demonstrated in cultured proximal tubular cells (Mellas et al., 1986).

The higher IGF-I mRNA levels after birth coincided with increased production of IGFBP- 3 mRNA, which we find in the mouse kidney exclusively located in the capillary system. Our precise cellular localization confirms the suggested expression sites of IGFBP-3 in rat

(Rabkin et al., 1995). Expression of IGFBP-3 in the capillary system may emphasize its main function in being a carrier protein for circulating IGFs. In addition, IGFBP-3 is involved in local actions of IGF, like vascularization (Nakao-Hayashi et al., 1992). The presence of IGFBP-3 in single cells of the mesenchyme, often accompanied by erythrocytes, fits with the assumption that these are endothelial cells, forming the capillary system of the fetal kidney (Pinson Hyink and Abrahamson, 1995). This defined localization correlates
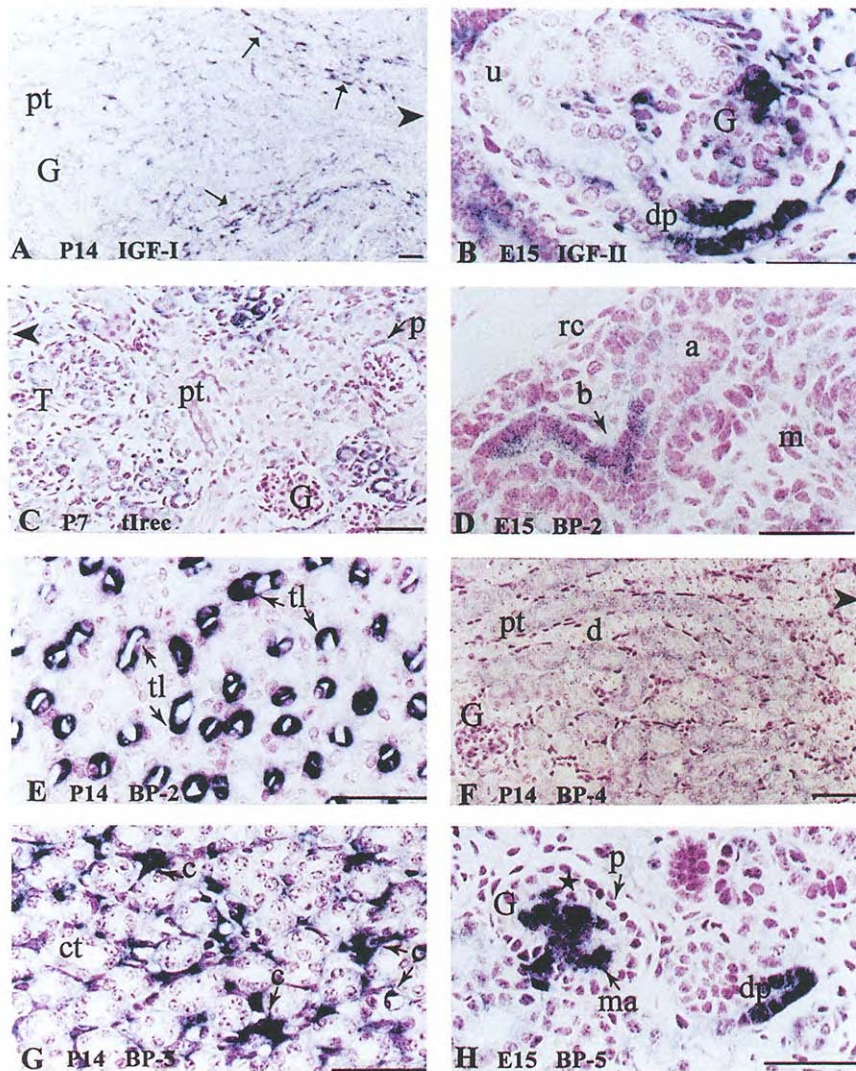
(Peri-)tubular expression

Fig. 4. Photomicrographs of in situ hybridization with DIG-labeled cRNA probes in developing and matured tubules in mouse kidney. (4A) IGF-I at day P14 in peritubular capillaries (→) in IC/OM, (4B) IGF-II at day E15, (4C) type I receptor at day P7 in IC/OM, (4D) IGFBP-2 at day E15, (4E) IGFBP-2 at day P14 in the medulla, (4F) IGFBP-4 at day P14, (4G) IGFBP-5 at day P14 in the medulla, (4H) IGFBP-5 at day E15. Abbreviations are: G, glomerulus; pt, proximal tubule; p, parietal epithelial cells; T, thick limb of Henle's loop; u, ureteric bud; dp, developing proximal tubule; d, distal tubule; ma, mesangial cells; ct, collecting tubule; c, peritubular capillary; rc, renal capsule; b, branching area of the ureteric bud; m, mesenchyme; tl, thin limb of Henle's loop; ✱, indicates erythrocytes present in capillary. Arrowhead points to the medullary region. Scale bar = 50 µM.

well with the observed expression in human nephrogenic mesenchyme (Matsell et al., 1994). Until maturation IGFBP-3 mRNA was expressed in the invading endothelial cells, forming the capillary loop of the developing glomerulus. Besides its possible role here in vascularization, IGFBP-3 might also act as a provider of IGFs to have their effect on the formation of the glomerular basement membrane (GBM) and the production of the extracellular matrix (ECM) in the mesangial cells (Pricci et al., 1996). IGFBP-3 was no longer expressed in the mature glomerulus, however during severe glomerulosclerosis in diabetic growth hormone transgenic mice IGFBP-3 mRNA is again expressed in the capillaries of the glomeruli (manuscript in preparation). This resumption of IGFBP-3 mRNA expression could be a result of elevated IGF-I levels (Clemmons et al., 1989; Bach and Jerums, 1990; Flyvbjerg et al., 1990) perhaps to enhance IGF action (Blum et al., 1989) in the expansion of mesangial ECM (Doi et al., 1989).

Expression patterns for IGFBP-5 mRNA in mouse were similar to those in man (Matsell et al., 1994; Chin

et al., 1994) and rat (Rabkin et al., 1995; Price et al., 1995). In the developing mouse kidney, IGFBP-5 mRNA co-localized with IGF-II mRNA at sites of cellular differentiation. This might imply a role for IGFBP-5 in potentiating IGF-II action as also has been described for osteoblasts in vitro (Andress and Birnbaum, 1992). Involvement in functional processes in the kidney is made likely by the increase in mRNA levels after birth. High IGFBP-5 mRNA expression in mesangial cells persisted in the mature glomerulus and expanded with the migration of the mesangial cells into the juxtaglomerular cells. Taking into account that IGFs have a stimulatory effect on the glomerular filtration rate (GFR) (Hirschberg et al., 1991), we might assume a mediating role for IGFBP-5 in this process. In vitro studies showed that ionic strength influences IGFBP-5 binding to ECM. Bound to ECM IGFBP-5 has a reduced affinity for IGFs, while being in solution its affinity increases (Jones et al., 1993). In this way IGFBP-5 can influence the availability of IGFs for their receptors and as a result the IGF actions in the vicinity of the glomerular filtration apparatus. The IGFBP-5 mRNA expression described in rat (Rabkin et al., 1995; Price et al., 1995) and human (Matsell et al., 1994; Chin

et al., 1994) medullary interstitium supports our defined localization in the peritubular capillaries of the medulla. The presence here of IGFBP-5 indicates an involvement in the IGF stimulated ion transport and exchange in the surrounding tubules (Zumkeller and Scofield, 1992).

In summary, we have shown that a combination of non radioactive in situ hybridization and quantitative RT-PCR results in a well defined localization and quantitation of mRNA expression. Our results demonstrate that during mouse kidney development each member of the IGF system has its specific mRNA expression pattern and altering mRNA levels. Distinct roles for each of them can be proposed in renal development and physiology and therefore our findings might be helpful to interpret observations of pathophysiological conditions.

Acknowledgements

Fig. 5. (5A) Quantitation profiles for mRNA of IGF-II and IGFBP-2 during mouse kidney development. Data represent the means ($n = 4$–6) ± S.E.M. ** Significant compared to E17 ($P < 0.02$); not significant compared to P3 ($P < 0.06$). * Significant compared to E17 ($P < 0.03$). # # Significant compared to P1 ($P < 0.02$). # Significant compared to E17 and P3 ($P < 0.05$). (5B). Quantitation profiles for mRNA of IGF-I, IGFBP-3 and -5 during mouse kidney development. Data represent the means ($n = 4$–6) ± S.E.M. ** Significant compared to E19 for all genes ($P < 0.02$).

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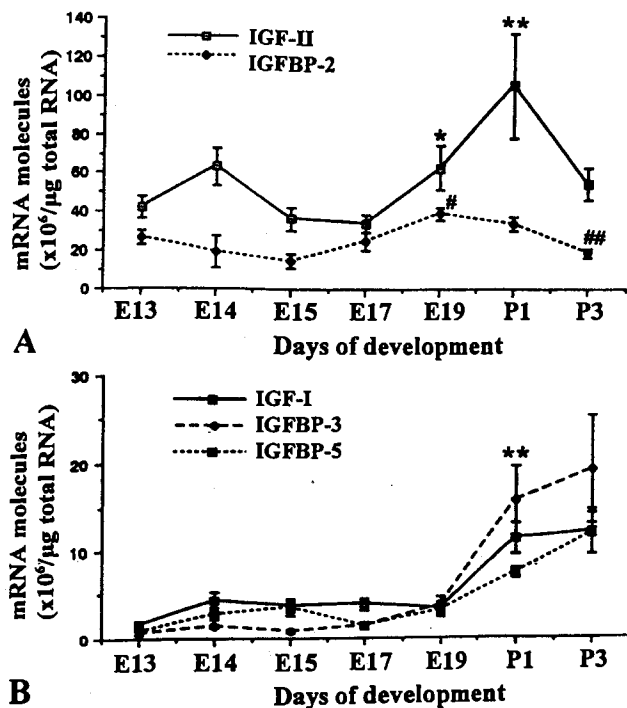
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