A Minimal Ksp-Cadherin Promoter Linked to a Green Fluorescent Protein Reporter Gene Exhibits Tissue-Specific Expression in the Developing Kidney and Genitourinary Tract

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Abstract. Ksp-cadherin is a unique, tissue-specific member of the cadherin family of cell adhesion molecules that is expressed exclusively in tubular epithelial cells in the kidney and developing genitourinary (GU) tract. Transgenic mice carrying 3425 bp of the Ksp-cadherin 5' flanking region linked to a lacZ reporter gene express β-galactosidase exclusively in the kidney, although the expression pattern is incomplete (Am J Physiol 277: F599–F610, 1999). To further define the region that mediates tissue-specific expression, transgenic mice carrying 1341 bp or 324 bp of the 5' flanking region linked to a green fluorescent protein (GFP) reporter gene were produced. Transgenic mice carrying 1341 bp of the 5' flanking region expressed GFP in all embryonic tissues that endogenously express Ksp-cadherin, including the ureteric bud, Wolffian duct, Müllerian duct, and developing tubules in the mesonephros and metanephros. In the adult kidney, GFP was highly expressed in thick ascending limbs of Henle’s loops and collecting ducts and weakly expressed in proximal tubules and Bowman’s capsules. Transgenic mice carrying 324 bp of the 5' flanking region exhibited expression exclusively in tubular epithelial cells in the developing kidney and GU tract. Immunoblot analysis showed that the expression of GFP was restricted to the kidney in adult mice. Taken together, these results demonstrate that 324 bp of the Ksp-cadherin 5' flanking region is sufficient to direct epithelial-specific expression in the developing kidney and GU tract. Transgenic mice that express GFP in the mesonephros, metanephros, ureteric bud, and sex ducts may be useful for cell lineage studies.

Ksp-cadherin (Cadherin 16) is a unique, tissue-specific member of the cadherin family of cell adhesion molecules that is expressed exclusively in tubular epithelial cells in the kidney and developing genitourinary (GU) tract (1,2). Cadherins are plasma membrane glycoproteins that mediate calcium-dependent cell-cell interactions and that play important roles in morphogenesis, cell differentiation, and signal transduction (3,4). Ksp-cadherin is a structurally distinct member of the family that has a unique tissue distribution. In adult mammals, Ksp-cadherin is found only in the kidney. Within the kidney, Ksp-cadherin is localized to the basolateral membrane of tubular epithelial cells comprising the nephrons and collecting ducts. Ksp-cadherin is expressed in all segments of the nephron, including the parietal epithelium of Bowman’s capsule, proximal tubules, loops of Henle, and distal tubules, but is not expressed in the glomerular tuft, blood vessels, or renal interstitial cells.

The expression of Ksp-cadherin in the kidney (metanephros) is developmentally regulated. Ksp-cadherin mRNA is first abundantly expressed in the mouse metanephros at E14.5 (Vanden Heuvel G and Igarashi P, unpublished observations). Expression increases during late gestation and remains high in the adult kidney. The protein is first expressed in developing nephrons after the S-shaped body stage (5). There is no expression in earlier nephric structures, including pretubular aggregates, renal vesicles, or comma-shaped bodies. Ksp-cadherin is expressed in the tubular portion of developing nephrons but is absent from the glomerular anlage. Ksp-cadherin is also expressed in the ureteric bud, which is an epithelial outgrowth from the mesonephric duct that is the anlage of the renal collecting system. Within the branching ureteric bud, the expression in maturing collecting ducts is higher than in the immature ampullae. Thus, the expression of Ksp-cadherin is differentiation-dependent as well as tissue-specific.

In addition to the metanephros, Ksp-cadherin is transiently expressed during embryogenesis in tubular epithelial cells of the developing GU tract. Ksp-cadherin transcripts have been detected by in situ hybridization in epithelial cells of the mesonephros, renal pelvis, and ureter (6). Transcripts are also expressed in the Wolffian (mesonephric) duct, which gives rise to the epididymis and vas deferens in males, and the Müllerian (paramesonephric) duct, which gives rise to the oviducts,
uterus, cervix, and vagina in females. A low level of mRNA expression has also been reported in the developing lung (6), but expression of Ksp-cadherin protein has not been detected in this tissue by antibody staining (R. Thomson, Yale Medical School, personal communication). The expression of Ksp-cadherin in the developing GU tract is transient, because there is no expression in the adult renal pelvis, ureter, or male and female genital tracts.

Although the biologic function of Ksp-cadherin remains unknown, it has proven to be a useful model of kidney-specific gene expression. To begin understanding the basis for its tissue-specific and developmentally regulated expression, we have previously cloned and characterized the promoter of the mouse Ksp-cadherin gene (7). The promoter is “TATA-less,” but it contains multiple GC-boxes and a CAAT box. The promoter also contains consensus recognition sites for several tissue-specific transcription factors, including basic-helix-loop-helix proteins, GATA factors, HNF-1, HNF-3, and C/EBP. Reporter gene assays in transiently transfected cells have shown that the Ksp-cadherin promoter can direct expression of a luciferase reporter gene in two renal epithelial cell lines (MDCK, mIMCD-3) but not in two mesenchymal cell lines (NIH3T3, MMR1), which indicates that the activity of the promoter is cell-specific (7). Transgenic mice carrying 3425 bp of the Ksp-cadherin 5' flanking region linked to a lacZ reporter gene express β-galactosidase exclusively in the kidney, verifying that the promoter is tissue-specific in vivo (8). However, the expression of the lacZ reporter gene is restricted to renal collecting ducts, whereas Ksp-cadherin is endogenously expressed in all nephron segments. In transgenic embryos, lacZ is expressed in the branching ureteric bud but is not expressed in developing nephrons. Taken together, these results demonstrate that 3425 bp of the Ksp-cadherin 5' flanking region directs tissue-specific expression in vitro and in vivo. However, this fragment of the promoter produces an incomplete expression pattern.

The minimal Ksp-cadherin promoter that is required for promoter activity in transfected renal epithelial cells has been defined by deletion analysis (7). MDCK cells and mIMCD-3 cells were transiently transfected with various lengths of Ksp-cadherin 5' flanking region linked to a luciferase reporter gene. Truncation of the promoter from -37 bp to +12 bp, GFP reporter gene, and bovine growth hormone polyadenylation signal was produced by digesting the plasmid BGZA (9) with NcoI and XhoI and replacing the 3.5-kb restriction fragment containing the lucZ gene and SV40 polyadenylation signal with the 1.1-kb NcoI/XhoI restriction fragment from pRES-EGFP (Clontech, Palo Alto, CA). A GFP reporter plasmid (pKsp1.3/BgEGFP) containing 1341 bp of the Ksp-cadherin 5' flanking region (nucleotides 2430–3770 of GenBank accession no. AF118228) was produced by digesting the plasmid pKsp(1268F)-lacZ with HindIII, isolating the 1.3-kb restriction fragment, end-filling with Klenow, and cloning the fragment into a unique Smal site upstream to the β-globin minimal promoter in BgEGFP. A GFP reporter plasmid (pKsp0.3/BgEGFP) containing 324 bp of the Ksp-cadherin 5' flanking region (nucleotides 3447–3770) was produced by digesting the plasmid pKsp(250F)-lacZ with HindIII, end-filling with Klenow, and then digesting with NheI. The 0.3-kb restriction fragment was isolated and cloned into unique XhoI and Smal sites upstream to the β-globin minimal promoter in BgEGFP. The sequences of the plasmid inserts were verified by DNA sequencing. The plasmids were tested by transient transfection into mIMCD-3 renal epithelial cells. Fluorescence microscopy revealed expression of GFP in transfected cells but not in untransfected cells (data not shown).

**Materials and Methods**

Mice (ICR or B6D2F1 hybrids) were obtained from Charles River Laboratories (Wilmington, MA) or The Jackson Laboratory (Bar Harbor, ME). Restriction endonucleases and DNA-modifying enzymes were from New England Biolabs (Beverly, MA) or Roche (Sommerville, NJ). Plasmid and genomic DNA preparation kits were from Qiagen (Valencia, CA). Lectins were from Vector Laboratories (Burlingame, CA) and EY Laboratories (San Mateo, CA). Other reagents were of molecular biologic grade from Sigma (St. Louis, MO), Promega (Madison, WI), or Roche. Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA).

**Plasmid Construction**

A reporter plasmid (BgEGFP) containing a human β-globin minimal promoter (~37 bp to +12 bp), GFP reporter gene, and bovine growth hormone polyadenylation signal was produced by digesting the plasmid pKsp(1268F)-lacZ (7) with HindIII, isolating the 1.3-kb restriction fragment, end-filling with Klenow, and cloning the fragment into a unique Smal site upstream to the β-globin minimal promoter in BgEGFP. A GFP reporter plasmid (pKsp1.3/BgEGFP) containing 1341 bp of the Ksp-cadherin 5' flanking region (nucleotides 2430–3770 of GenBank accession no. AF118228) was produced by digesting the plasmid pKsp(1268F)-lacZ (7) with HindIII, isolating the 1.3-kb restriction fragment, end-filling with Klenow, and cloning the fragment into a unique Smal site upstream to the β-globin minimal promoter in BgEGFP. A GFP reporter plasmid (pKsp0.3/BgEGFP) containing 324 bp of the Ksp-cadherin 5' flanking region (nucleotides 3447–3770) was produced by digesting the plasmid pKsp(250F)-lacZ (7) with HindIII, end-filling with Klenow, and then digesting with NheI. The 0.3-kb restriction fragment was isolated and cloned into unique XhoI and Smal sites upstream to the β-globin minimal promoter in BgEGFP. The sequences of the plasmid inserts were verified by DNA sequencing. The plasmids were tested by transient transfection into mIMCD-3 renal epithelial cells. Fluorescence microscopy revealed expression of GFP in transfected cells but not in untransfected cells (data not shown).

**Generation of Transgenic Mice**

Transgene fragments were isolated from vector sequences by digestion with NotI and XhoI followed by agarose gel electrophoresis, electroelution, and purification by anion-exchange chromatography (Elutip-d; Schleicher & Schuell, Keene, NH). The purified DNA was concentrated in Microcon-30 filters (Millipore, Bedford, MA), resuspended at a concentration of 2 to 3 μg/ml in microinjection buffer (10 mM Tris-Cl [pH 7.40], 0.25 mM EDTA), and sterilized by filtration through 0.2-μm filters. Transgene DNA was microinjected into the pronuclei of fertilized oocytes as described previously (8,9). Fertilized oocytes were from B6D2F1 crosses or ICR donors. Some microinjections were performed by the UT Southwestern Transgenic Mouse Core Facility. Microinjected embryos were transferred into the oviducts of pseudopregnant foster mothers and were permitted to develop to term. Transgenic progeny were identified by PCR analysis of tail DNA. The primers used for amplification of the GFP gene were


**GFP Expression in Developing Kidney**

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controls, samples from nontransgenic littermates were photographed using Spot or Zeiss digital cameras and a Power Macintosh computer equipped with Adobe Photoshop and Openlab software. As negative controls, specimens were cryoprotected overnight in 30% sucrose, embedded in OCT, frozen in isopentane, and sectioned at 5 to 10 μm. Sections were mounted with Vectashield. Photomicrographs were obtained using a Leica Frigocut 2800N cryostat. Sections were mounted with Vectashield and visualized using a Nikon TE300 inverted microscope equipped with a standard FITC filter set (excitation, 460 to 500 nm; dichroic mirror, 505 nm; emission, 510 to 560 nm). Embryos were obtained by Caesarean section from timed-pregnant mice at E9.5 to E17.5 and were hand-dissected to expose the kidney and GU tract before examination. Thin sections were prepared from tissues and embryos by perfusion-fixation and/or immersion for 1 h in phosphate-buffered saline (PBS) containing 4% paraformaldehyde. Specimens were cryoprotected overnight in 30% sucrose, embedded in OCT, frozen in isopentane, and sectioned at 5 to 10 μm using a Leica Frigocut 2800N cryostat. Sections were mounted with aqueous medium (Vectashield) and visualized using a Nikon TE300 or Zeiss Axioplan 2 microscope. Photomicrographs were obtained using Spot or Zeiss digital cameras and a Power Macintosh computer equipped with Adobe Photoshop and Openlab software. As negative controls, samples from nontransgenic littermates were photographed under identical exposure conditions.

**Green Fluorescent Protein**

The expression of GFP was examined by fluorescence microscopy of fresh, unfixed tissues and embryos. Tissues were sectioned at 50 to 100 μm using a Vibratome 1500 system (St. Louis, MO) and were visualized under epifluorescence illumination using a Nikon TE300 inverted microscope equipped with a standard FITC filter set (excitation, 460 to 500 nm; dichroic mirror, 505 nm; emission, 510 to 560 nm). Embryos were obtained by Caesarean section from timed-pregnant mice at E9.5 to E17.5 and were hand-dissected to expose the kidney and GU tract before examination. Thin sections were prepared from tissues and embryos by perfusion-fixation and/or immersion for 1 h in phosphate-buffered saline (PBS) containing 4% paraformaldehyde. Specimens were cryoprotected overnight in 30% sucrose, embedded in OCT, frozen in isopentane, and sectioned at 5 to 10 μm using a Leica Frigocut 2800N cryostat. Sections were mounted with aqueous medium ( Vectashield) and visualized using a Nikon TE300 or Zeiss Axioplan 2 microscope. Photomicrographs were obtained using Spot or Zeiss digital cameras and a Power Macintosh computer equipped with Adobe Photoshop and Openlab software. As negative controls, samples from nontransgenic littermates were photographed under identical exposure conditions.

**Immunostaining and Lectin Staining**

Immunostaining and indirect immunofluorescence were performed as described previously (8). Five- to ten-micrometer-thick cryosections were prepared from formalddehyde-fixed tissues, mounted, and incubated with primary antibody for 1 h at room temperature. An affinity-purified rabbit antibody to green fluorescent protein (generous gift from Dr. Pam Silver, Harvard Medical School, Cambridge, MA) was used at a dilution of 1:2000 (11). A rabbit antibody to mouse Tamm-Horsfall protein (generous gift from Dr. John Hoyer, University of Pennsylvania, Philadelphia, PA) was used at a dilution of 1:2000 (12). Sections were then washed with PBS and incubated with a 1:200 dilution of Alexa Fluor 488–coupled or Alexa Fluor 594–coupled goat anti-rabbit IgG (Molecular Probes, Eugene, OR). Stained sections were mounted with Vectashield and examined using a Nikon TE300 or Zeiss Axioplan 2 microscope. Lectin staining was performed on 10-μm-thick cryosections of kidneys and embryos as described previously (8). Collecting ducts and ureteric buds were labeled by staining with TRITC-conjugated Doli-chos biflorus agglutinin (DBA). Proximal tubules were labeled by staining with TRITC-coupled Lotus tetragonolobus agglutinin (LTA). After washing with PBS containing 0.1% bovine serum albumin, the sections were postfixed in PBS containing 4% paraformaldehyde, rinsed in PBS, and then mounted with Vectashield. Photomicrographs were obtained as described above. Images of double-labeled sections were merged using Openlab or Adobe Photoshop software.

**In Situ Hybridization**

Radioactive in situ hybridization was performed on paraffin-embedded sections using 35S-radiolabeled RNA probes as described previously (13). Antisense and sense riboprobes were transcribed from a 900-bp mouse Ksp-cadherin cDNA cloned in the plasmid pCR2.1. After hybridization and washing, the slides were dipped in emulsion, exposed, and developed in Kodak D19. Photomicrographs were taken with a Leica M420 microscope equipped with a darkfield condenser.

**Organ Culture**

Metanephric organ culture was performed using an adaptation of a previously described method (14). Metanephroi were isolated from embryos at E12.5 and were placed on transparent Cyclopore filters. Metanephroi were cultured for up to 7 d at the air-medium interface in DMEM/Ham’s F12 containing 10% fetal calf serum. Cultures were photographed under phase-contrast and epifluorescence illumination using a Nikon TE300 inverted microscope.

**Immunoblot Analyses**

Tissue samples were homogenized for 20 s in 5 volumes (wt/vol) of PBS containing protease inhibitors (Complete Mini Protease Inhibitor Cocktail; Roche). Samples were centrifuged (10 min; 16000 × g), and the supernatant was frozen for 2 h at −20°C. After thawing, centrifugation was repeated. Protein concentration was determined using the Coomassie protein reagent (Pierce, Rockford, IL) with bovine serum albumin as the standard. Protein samples (100 μg) were reduced (100 mM DTT; 70°C; 10 min), analyzed by SDS-PAGE using 4 to 12% Bis-Tris gels (Invitrogen, Carlsbad, CA), and electrophoretically transferred to nitrocellulose membranes (Hybond-C, Amersham, Gaithersburg, MD). Membranes were blocked in PBS containing 5% nonfat dry milk and 0.05% Tween 20, and they were then incubated in the same solution containing rabbit anti-GFP antibody (1:1000), monoclonal mouse anti-Ksp-cadherin antibody (1:1000; generous gift from Dr. Brent Thomson, Yale Medical School, New Haven, CT), or rabbit anti-LRP85 antibody (1:1000; generous gift from Dr. Joachim Herz, University of Texas Southwestern, Dallas, Texas) (15). Bound antibodies were detected with peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG (1:2000) and enhanced chemiluminescence (SuperSignal, Pierce).

**Results**

**Generation of Transgenic Mice**

To narrow down the region of the 5' flanking region that was responsible for tissue-specific expression of the Ksp-cadherin gene, genomic fragments containing 1341 bp or 324 bp of the 5' flanking region cloned upstream of a β-globin minimal promoter and a green fluorescent protein (GFP) reporter gene were analyzed in transgenic mice. Figure 1 shows the structures of the transgenes. The β-globin minimal promoter is transcriptionally inactive in transgenic mice in the absence of added enhancers (16). Therefore, if the test fragments contain tissue-specific regulatory elements that can activate the heterologous β-globin promoter, tissue-specific expression of the GFP reporter gene will be observed. The test fragments also contain the Ksp-cadherin transcription start site; therefore, activation of the homologous promoter will also be detected in this assay. GFP, an intrinsically fluorescent protein from A. victoria, was selected as the reporter because it can be visual-
To compare the expression of GFP with the endogenous expression of Ksp-cadherin, RNA in situ hybridization was performed. Paraffin-embedded sections of adult mouse kidney were hybridized with 35S-labeled riboprobes for Ksp-cadherin. Figure 2D shows darkfield illumination of the kidney after hybridization with an antisense Ksp-cadherin riboprobe. Similar to the expression of GFP, the expression of Ksp-cadherin mRNA was detected throughout the kidney, especially in the outer medulla, but not in the region of the vascular bundles. Hybridization with a sense Ksp-cadherin riboprobe produced no significant signal, indicating that the hybridization to the antisense riboprobe was specific (Figure 2E). Higher magnification images of the renal medulla showed expression in tubular profiles (Figure 2F). The pattern of hybridization was consistent with the high expression of Ksp-cadherin protein and mRNA in thick ascending limbs of Henle’s loops and collecting ducts (5, 6).

Examination of thin cryosections of a transgenic kidney under fluorescence microscopy confirmed that GFP was expressed in tubular epithelial cells. In the outer medulla, GFP was highly expressed in thick ascending limbs of Henle’s loops and medullary collecting ducts but was absent from blood vessels within the vascular bundles (Figure 2G). In the renal cortex, the overall level of expression was lower than in the medulla (Figure 2H). GFP was expressed at high levels in cortical thick ascending limbs of Henle’s loops and cortical collecting ducts and was weakly expressed in proximal tubules and the parietal epithelia of Bowman’s capsules. No fluorescence signal was detected in glomerular tufts or in the kidney from a nontransgenic littermate (Figure 2I). Four independent founders that expressed the Ksp1.3/BgEGFP transgene were produced. Three founders expressed GFP in the pattern described above, and one exhibited partial expression in the renal medulla.

Expression of the Ksp1.3/BgEGFP Transgene in Renal Tubules

To verify that the green fluorescence signal was due to the expression of GFP, thin sections of the kidneys from Ksp1.3/BgEGFP hemizygous mice were immunostained with an antibody directed against the protein (11). Figure 3A shows a section of the renal cortex from a transgenic mouse stained with a GFP primary antibody and an Alexa Fluor 488–coupled secondary antibody. Only tubular epithelial cells stained positive with the antibody. There was no staining of glomerular tufts, blood vessels, or renal interstitial cells. The kidney from a nontransgenic littermate showed no significant staining under identical conditions (Figure 3D), indicating that the antibody staining was specific. To identify the GFP-positive tubules in panel A, the section was costained with TRITC-coupled Lotus tetragonolobus (LTA), a lectin that specifically labels proximal tubules (18). The merged image (Figure 3C) shows expression of GFP in LTA-positive proximal tubules. Proximal tubules in the renal cortex expressed moderate levels of GFP, whereas proximal tubules in the outer medulla, which probably represent S3 segments, showed minimal expression (Figure 2G).

The highest expression of GFP was in the loops of Henle and
collecting ducts. Figures 3E and 3F show the renal medulla from a transgenic mouse costained with a GFP antibody and TRITC-coupled Dolichos biflorus agglutinin (DBA), a lectin that specifically labels collecting ducts (18). The merged image (Figure 3F) shows expression of GFP in DBA-positive collecting ducts. Figures 3G through 3I show GFP fluorescence in the outer medulla and staining with an antibody to Tamm-Horsfall protein (THP), which is expressed in thick ascending limbs of Henle’s loops and distal tubules (19). The merged image (Figure 3I) shows coexpression of GFP and THP in thick ascending limbs of Henle’s loops. Coexpression was also observed in THP-positive distal tubules in the superficial cortex (not shown). Taken together, these results demonstrated that the expression of the Ksp1.3/BgEGFP transgene in adult mice was restricted to renal tubular epithelial cells. The transgene was expressed in Bowman’s capsules, proximal tubules, loops of Henle, distal tubules, and collecting ducts, which was identical to the expression of the endogenous Ksp-cadherin protein described previously (1,5,8).

Expression of the Ksp1.3/BgEGFP Transgene in Mouse Embryos

The expression of Ksp-cadherin in the kidney and GU tract is developmentally regulated. To test whether the expression of the Ksp1.3/BgEGFP transgene was regulated similarly, transgenic embryos were examined at different gestational ages. Figure 4A shows fluorescence microscopy of a transgenic embryo at E15.5, a stage at which Ksp-cadherin is expressed in the kidney (metanephros), ureter, and sex ducts. Green fluorescence signal was observed in the metanephros, ureter, and Wolffian duct but not in the testis or surrounding tissues. No significant fluorescence was observed in the kidney from a nontransgenic littermate (Figure 4B). To verify the expression

Table 1. Summary of the patterns of expression of the transgenes and comparison with the endogenous expression pattern of Ksp-cadherin as well as the expression of the Ksp3.3/nLacZ transgene

<table>
<thead>
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<th>Transgene</th>
<th>Ksp3.3/nLacZa</th>
<th>Ksp1.3/BgEGFP</th>
<th>Ksp0.3/BgEGFP</th>
<th>Endogenous Expression b</th>
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<tr>
<td>Independent founders (n)</td>
<td>ND</td>
<td>9</td>
<td>9</td>
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<tr>
<td>Expressed transgene (n)</td>
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<td>4d</td>
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a ND, not determined.
b From reference 8.
c From references 1, 5, 6, 8.
d One with partial expression.
e One with ectopic expression in lung and skin.
Figure 2. Expression of green fluorescent protein (GFP) in Ksp1.3/BgEGFP transgenic mice. (A) Fluorescence microscopy of a vibratome section (100 μm) of the kidney from a transgenic mouse shows high levels of GFP fluorescence in the outer medulla (om) and lower levels of fluorescence in the cortex (co) and inner medulla (im). Vascular bundles (arrowheads) show less fluorescence than surrounding tubules. (B) A vibratome section of the kidney from a nontransgenic mouse photographed under identical conditions shows no significant fluorescence. (C) The liver (li) from a transgenic mouse also shows no fluorescence. (D) In situ hybridization of Ksp-cadherin mRNA transcripts in the adult mouse kidney. Hybridization with an antisense riboprobe and visualization under darkfield illumination shows high expression of Ksp-cadherin in the outer medulla (om) and lower expression in the cortex (co) and inner medulla (im). There is no expression in vascular bundles (arrowheads). (E) Hybridization of the kidney with a sense riboprobe shows no significant signal. (F) Higher magnification image of the renal medulla after hybridization with an antisense riboprobe shows expression of Ksp-cadherin in collecting ducts (cd) in the inner medulla (im) but no expression in vascular bundles (arrowheads). (G) Fluorescence microscopy of a cryosection (10 μm) of the outer medulla from a
of GFP, transgenic embryos were sectioned and stained with a specific antibody. As shown in Figure 4C, immunostaining was restricted to the developing kidney (metanephros) and GU tract. There was no expression of GFP in any of the surrounding tissues, including the liver, intestine, and rectum, which verified that the expression of the transgene was tissue-specific.

Examination of embryos at earlier stages of development showed that GFP was not expressed before E10.5 (data not shown). At E11.5, GFP was expressed in the mesonephros, which is a transient excretory organ in mammals, and in the mesonephric (Wolffian) duct (Figure 4D). There was no expression in the surrounding tissues including lung, liver, heart, and stomach. A higher magnification image (Figure 4E) showed that GFP was expressed in epithelial cells comprising the mesonephric tubules and Wolffian duct. At E12.5, GFP was expressed within the developing metanephros in epithelial cells of the branching ureteric bud and developing tubules (Figure 4F). Colabeling with LTA, THP, and DBA showed that the transgene was expressed at high levels in the branching ureteric bud and developing collecting ducts and at lower levels in developing loops of Henle and proximal tubules (data not shown). Similar to the endogenous Ksp-cadherin gene, which is expressed in developing nephrons only after the S-shaped body stage, GFP was not expressed in metanephric mesenchyme, S-shaped bodies, or earlier nephric structures. To determine whether the expression of GFP could be detected in metanephric organ culture, metanephroi were removed from transgenic organ culture. GFP was shown to be restricted to tubular epithelial cells. However, in contrast to transgenes containing the 1341-bp fragment of the 5’ flanking region, which were expressed uniformly in renal tubules (except for one founder that exhibited partial expression), all the founders carrying the 324-bp fragment exhibited variegated expression in which the transgene was expressed in some, but not all, the cells. Figures 5G through 5I show that GFP was expressed in a variegated pattern in developing loops of Henle, collecting ducts, and proximal tubules. In addition to variegated expression, one founder exhibited ectopic expression in the developing lung and skin (data not shown).

Expression of the Ksp0.3/BgEGFP Transgene in Adult Mice

To verify the tissue-specificity of the Ksp0.3/BgEGFP transgene, tissue homogenates were prepared from the kidney and various organs of adult transgenic mice and were subjected to immunoblot analyses using an anti-GFP antibody. Figure 6A shows that the antibody recognized a protein of the expected size (27 kDa) that was only detected in the kidney. Identical results were obtained in mice derived from a second, independent founder (Figure 6B). Immunoblot analysis using a monoclonal antibody to Ksp-cadherin showed that the pattern of expression of GFP was identical to the endogenous expression of Ksp-cadherin, which was also only detected in the kidney (Figure 6C). In contrast, Figure 6D shows that the small fragment of the LDL receptor-related protein (LRP-85) was

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Expression of the Ksp0.3/BgEGFP Transgene in Embryos and Newborn Mice

Next, we expressed transgenes containing a 324-bp fragment of the 5’ flanking region of the Ksp-cadherin gene. This region contains the 250-bp promoter that had previously been shown to be sufficient for promoter activity in transiently transfected renal epithelial cells (7). Transgenic mice carrying the 324-bp fragment linked to a GFP reporter gene were produced by pronuclear microinjection, and the expression of GFP in the hemizygous progeny was examined by fluorescence microscopy and immunostaining. Figures 5A and 5B show fluorescence microscopy of the kidneys from newborn mice derived from two independent founders. Both mice exhibited GFP fluorescence in the kidney, whereas no fluorescence was detectable in the kidneys from nontransgenic littermates (not shown). Moreover, no fluorescence was observed in the heart from a transgenic mouse (Figure 5C), indicating that the expression of GFP was kidney-specific. A higher magnification image of the renal medulla from a transgenic mouse shows that GFP was expressed in collecting ducts (Figure 5D). Figures 5E and 5F show the expression of GFP in a transgenic embryo at E17.5. GFP was expressed only in the metanephros and not in the stomach, pancreas, intestine, and adrenal gland, which verified that the expression was tissue-specific in the developing embryo. Within the metanephros, the expression of GFP was restricted to tubular epithelial cells. However, in contrast to transgenes containing the 1341-bp fragment of the 5’flanking region, which were expressed uniformly in renal tubules (except for one founder that exhibited partial expression), all the founders carrying the 324-bp fragment exhibited variegated expression in which the transgene was expressed in some, but not all, the cells. Figures 5G through 5I show that GFP was expressed in a variegated pattern in developing loops of Henle, collecting ducts, and proximal tubules. In addition to variegated expression, one founder exhibited ectopic expression in the developing lung and skin (data not shown).
Figure 3. Expression of the Ksp1.3/BgEGFP transgene in renal tubules. (A through C) Renal cortex from a transgenic mouse stained with a primary antibody to GFP and an Alexa Fluor 488-coupled secondary antibody (A) and colabeled with TRITC-coupled *Lotus tetragonolobus* agglutinin (LTA) (B). The merged image (C) shows that GFP (green) is expressed in proximal tubules (pt), which stain positive with LTA (red). GFP is also highly expressed in collecting ducts (cd) and thick ascending limbs of Henle’s loops (arrowhead) that do not stain with LTA, but there is no expression in glomeruli (gl). (D) The renal cortex from a nontransgenic mouse shows no significant GFP immunostaining under identical conditions. (E and F) Renal outer medulla from a transgenic mouse stained with a primary antibody to GFP and an Alexa Fluor 488-coupled secondary antibody (E) and co-labeled with TRITC-coupled *Dolichos biflorus* agglutinin (DBA) (F). The merged image (F) shows that GFP (green) is expressed in collecting ducts (cd), which stain positive with DBA (red). GFP is also highly expressed in thick ascending limbs of Henle’s loops (arrowheads) that do not stain with DBA. The low expression in proximal tubules (pt) in the outer medulla is not visible under these exposure conditions. (G through I) Renal outer medulla from a transgenic mouse visualized by fluorescence microscopy (G) and stained with a primary antibody to Tamm-Horsfall protein (THP) and Alexa Fluor 594-coupled secondary antibody (H). The merged image (I) shows that GFP (green) is highly expressed in thick ascending limbs of Henle’s loops (tal), which also express THP (red). Arrowhead indicates GFP expression in a collecting duct. Scale bars: 50 μm.
Figure 4. Expression of GFP in Ksp1.3/BgEGFP transgenic embryos. (A) Fluorescence microscopy of a transgenic embryo at E15.5 after removal of the overlying skin, liver, and intestine. GFP fluorescence is present in the kidney (ki), ureter (ur), and Wolffian duct (wd) but not in the testis (te) or surrounding tissues. (B) A non-transgenic embryo photographed under identical conditions shows no significant fluorescence. (C) Sagittal section of a transgenic embryo at E15.5 stained with a primary antibody to GFP and an Alexa Fluor 594-coupled secondary antibody. GFP is expressed in the kidney (ki), ureter (ur), and Wolffian duct (wd) but not in the liver (li), intestine (in), or rectum (re). (D and E) Sagittal section of a transgenic embryo at E11.5 stained with an antibody to GFP shows expression in the Wolffian duct (wd) and mesonephric tubules (me) but not in the heart (he), liver (li), stomach (st), or lung (lu). (F) The metanephros from a transgenic embryo at E15.5 stained with an antibody to GFP shows expression in the branching ureteric bud (ub) and developing tubules (tu). There is no expression in S-shaped bodies (sb) or metanephric mesenchyme (mes). (G and H) Fluorescence (G) and phase-contrast (H) images of an E12.5 metanephros after 3 d in organ culture. Arrowheads indicate GFP fluorescence in the branching ureteric bud. (I) Immunostaining of a transgenic embryo at E15.5 shows expression of GFP in the developing ureter (ur), Wolffian duct (wd), and Müllerian duct (md). Scale bars: 500 μm in A, B, C, and D; 100 μm in E, F, I; 200 μm in G and H.
Figure 5. Expression of GFP in Ksp0.3/BgEGFP transgenic mice. (A and B) GFP fluorescence in the kidneys of newborn mice derived from two independent founders (lines 41 and 23). (C) The heart (he) from a transgenic mouse photographed under identical conditions shows no fluorescence. (D) Higher magnification image of the renal medulla from a transgenic mouse shows GFP fluorescence in collecting ducts (arrowheads). (E and F) Indirect immunofluorescence (E) and phase-contrast (F) images of a transgenic embryo at E17.5 stained with an antibody to GFP and Alexa Fluor 594–coupled secondary antibody. GFP is expressed in the kidney (ki) but not in the stomach (st), intestine (in), pancreas (pa), or adrenal gland (ad). (G) GFP fluorescence (green) and THP immunostaining (red) of an E17.5 metanephros shows expression of GFP in developing loops of Henle (hl). Note the variegated expression pattern. (H) GFP immunostaining (green) and DBA staining (red) of an E17.5 metanephros shows variegated expression of GFP in developing collecting ducts (cd). (I) GFP immunostaining (green) and LTA staining (red) of an E17.5 metanephros shows variegated expression of GFP in developing proximal tubules (pt). Scale bars: 500 μm in A, B, and C; 200 μm in D, E, and F; 50 μm in G, H, and I.
Figure 6. Immunoblot analyses of GFP expression in Ksp0.3/BgEGFP transgenic mice. 100 μg of proteins from brain (Br, lane 1), heart (He, lane 2), lung (Lu, lane 3), liver (Li, lane 4), kidney (Ki, lane 5), spleen (Sp, lane 6), stomach (St, lane 7), and skeletal muscle (Sk, lane 8) were separated by 4 to 12% SDS-PAGE and transferred to nitrocellulose. Immunoblot analyses were performed using antibodies directed against GFP (A and B), Ksp-cadherin (C), and the smaller fragment of LRP (LRP-85) (D). Panels A and B contain samples derived from two independent founders (lines 20 and 23, respectively). Positions of molecular weight standards are shown on the left.

detected in all tissues. These results confirmed that the expression of the Ksp0.3/BgEGFP transgene was kidney-specific in the adult. Table 1 summarizes the patterns of expression of the Ksp-cadherin gene as well as the expression of the Ksp3.3/LacZ transgene reported previously (8).

Discussion

Previous studies have shown that a 3425-bp fragment of the 5′ flanking region containing the promoter of the Ksp-cadherin gene can direct kidney-specific expression of a lacZ reporter gene in transgenic mice but produces an incomplete expression pattern. We now show that transgenes containing 1341 bp or 324 bp of the 5′ flanking region linked to a GFP reporter gene are also exclusively expressed in tubular epithelial cells in the kidney and developing GU tract. The 1341-bp fragment of the 5′ flanking region recapitulates the complete expression pattern of the Ksp-cadherin gene. In adult mice, transgenes containing the 1341-bp fragment are expressed exclusively in kidney tubules. Like the endogenous Ksp-cadherin gene, the transgenes are highly expressed in loops of Henle and collecting ducts and weakly expressed in Bowman’s capsule and proximal tubules. Similar expression patterns are observed in multiple, independent founders; tissue-specific expression is therefore due to elements contained within the transgenes and is not due to DNA flanking the transgene integration sites.

In addition to expression in the adult kidney, the Ksp1.3/BgEGFP transgene is also expressed in the developing kidney and GU tract. The transgene is expressed in tubular epithelial cells of the mesonephros, metanephros, ureteric bud, and sex ducts. Expression in the developing ureteric bud and sex ducts is transient, because there was no expression in the adult ureter or genital tracts. In the developing metanephros, the transgene is expressed in the branching ureteric bud and in developing nephrons after the S-shaped body stage. There is no expression in early nephric structures, including condensed mesenchyme, renal vesicles, and comma-shaped bodies. The expression in the developing metanephros, mesonephros, and sex ducts is identical to the expression of the Ksp-cadherin mRNA and protein reported previously (5,6). Therefore, the 1341-bp fragment of 5′ flanking region contains regulatory elements that are sufficient to completely recapitulate the tissue-specific and developmentally regulated expression of the Ksp-cadherin gene.

Although other tissue-specific promoters have been shown to direct transgene expression in the ureteric bud and renal collecting system (e.g., Pax-2, aquaporin-2, Hoxb7), the 5′ flanking region of the Ksp-cadherin gene is the first genomic region that has been shown to direct tissue-specific expression in developing renal tubules in the mesonephros and metanephros. The 5′ flanking region should be useful for expressing any gene of interest in kidney tubules in transgenic mice. One example of this type of experiment is described in a companion article (20), in which the 1341-bp fragment was used to express Cre recombinase in the developing kidney and GU tract. Interestingly, the 1341-bp fragment of the Ksp-cadherin 5′ flanking region produces a more complete expression pattern than the longer 3425-bp fragment described previously (8). Transgenes containing the 3425-bp fragment are expressed in a tissue-specific pattern, but the expression is incomplete and confined to collecting ducts and ureteric bud. The 3425-bp fragment may contain cis-acting element(s) that inhibit transgene expression in mesonephric tubules and developing nephrons.

Ksp0.3/BgEGFP transgenic mice carrying a 324-bp fragment of the 5′ flanking region also express GFP only in the developing kidney and GU tract. However, caution is necessary in the interpretation of the results with the 324-bp promoter. In contrast to the uniform expression produced by the 1341-bp fragment, the 324-bp fragment produces a variegated expression pattern. Variegated expression is frequently observed in transgenic mice and reflects transgene silencing in a fraction of the cells. The mechanism is poorly understood, but it is believed to involve epigenetic modification of the transgene due to chromosomal position effects (21). The 324-bp region may lack an insulator element or locus control region that mitigates against variegating position effects. Despite the presence of variegating position effects, our studies show that transgenes containing 324 bp of the Ksp-cadherin 5′ flanking region of Ksp3.3/nLacZ transgene reported previously (8).

region are expressed exclusively in the kidney and GU tract in developing embryos. Immunoblot analysis verifies that the expression of the transgene is kidney-specific in adult mice. Taken together, these results demonstrate that a 324-bp fragment of Ksp-cadherin 5′ flanking region linked to a β-globin minimal promoter and GFP reporter gene exhibits tissue-specific expression in transgenic mice.

The 324-bp fragment is the shortest genomic fragment that has been demonstrated to have kidney-specific activity in transgenic mice. Because of the variegated expression pattern, further deletion analysis of the region was not attempted. Future experiments will be directed at identifying the transcription factors that bind to the 324-bp fragment and that mediate tissue-specific gene expression. The 324-bp region contains a (CA)n microsatellite repeat that is not conserved in the human promoter and is therefore unlikely to be essential for tissue-specific expression. A 120-bp sequence that is >80% identical between mouse and human is more likely to contain essential elements. Recent studies have shown that this region contains a functional binding site for hepatocyte nuclear factor 1 (HNF-1), a homeodomain protein that is involved in kidney-specific gene expression (22).

Although GFP has been used as a reporter gene in transgenic mice, there are relatively few descriptions of its use in the kidney. Transgenes containing GFP under the regulation of the renin promoter are expressed in juxtaglomerular cells and several extrarenal tissues (23). Transgenes containing the Hoxb7 promoter and GFP reporter gene are expressed in the ureteric bud but also in the brain and snout (24). CX/GFP transgenes containing a cytomegalovirus enhancer and β-actin/β-globin promoter are expressed in podocytes as well as skeletal muscle, pancreas, and heart (25). Transgenes containing the THP promoter linked to a GFP reporter gene exhibit kidney-specific expression in thick ascending limbs of Henle’s loops and early distal tubules (26). The current study further supports the utility of GFP as a reporter gene in the kidney. Expression can be detected by fluorescence microscopy of unfixed tissues, cultured organs, or formaldehyde-fixed tissues as well as by immunostaining. Because the expression of GFP can be detected in living cells, Ksp1.3/BgEGFP transgenic mice may be useful for future cell lineage studies. For example, pluripotent stem cells isolated from Ksp1.3/BgEGFP transgenic mice could be induced to differentiate, and the expression of GFP could be monitored as an indicator of tubular epithelial differentiation.

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