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Re-expression of the developmental gene *Pax-2* during experimental acute tubular necrosis in mice[1]

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Background. The transcription factor *Pax-2* is known to play a key regulatory role during embryonic development of the nervous and excretory systems in mammals and flies. During mouse kidney development, *Pax-2* is expressed in the undifferentiated mesenchyme in response to ureter induction and continues to be expressed in the developing comma- and s-shaped bodies. These structures harbor the immediate precursors of the proximal tubular epithelial cells. *Pax-2* expression is down-regulated as the differentiation of the functional units of the nephron proceeds. In the adult mammalian kidney, the *Pax-2* protein is detectable exclusively in the epithelium of the collecting ducts. We sought to test the hypothesis that tissue regeneration is characterized by re-expression of developmentally important regulatory genes such as *Pax-2*.

Methods. The expression pattern of *Pax-2* in kidneys after experimentally-induced acute tubular necrosis caused by intraperitoneally injected folic acid in mice was tested by indirect immunofluorescence, Western blotting, reverse transcriptase-polymerase chain reaction, and *in situ* hybridization analysis.

Results. A transient, temporally and locally restricted re-expression of *Pax-2* in regenerating proximal tubular epithelial cells was observed following kidney damage.

Conclusions. These data indicate that during the regeneration processes, developmental paradigms may be recapitulated in order to restore mature kidney function.

Acute tubular necrosis, with prerenal disease, is one of the most common causes of acute renal failure, accounting for two thirds of intrinsic causes [1]. The molecular basis of the events leading to tubular regeneration after acute tubular necrosis (ATN) is not understood. It has been suggested that regeneration processes may recapitulate developmental paradigms in order to restore organ or tissue function [2, 3]. This hypothesis is testable in part by examination of the similarities between developmental and regenerative processes on a molecular level seen during experimental ATN.

The adult tubular epithelium has a potential of regeneration following damage. During ATN, normally quiescent cells undergo dedifferentiation and reobtain their potential to divide after enhancing their DNA synthesis [4, 5]. Following proliferation, the new cells then differentiate to restore the functional integrity of the nephron.

Many genes have been shown to be modulated in response to kidney damage. The expression of the growth factors *HGF* and *IGF-1* is up-regulated after artificial kidney damage, whereas *EGF* expression is down-regulated. Other genes that are up-regulated after ATN are the immediate early genes, such as *c-fos*, *c-myc*, *c-jun*, or *EGR-1* [reviewed in 6]. In contrast, *Kid-1*, a zinc finger gene, is expressed solely in adult kidneys and not in embryonic kidneys [7]. Because the expression of *Kid-1* is lost in proximal tubules of folic acid-treated animals [7], its down-regulation may reflect a functional stage similar to early kidney development.

One example demonstrating the biological importance of biphasic protein expression during development and adulthood includes the expression of *bcl-2* and *bax* known for their antiapoptotic and proapoptotic roles, respectively, during early metanephric development [8, 9]. In adulthood, these genes have been shown to be re-expressed in proximal tubular cells after ischemic damage [10]. Vimentin is an intermediate filament and a marker of undifferentiated mesenchymal cells. It is not present in the healthy adult tubule but its re-expression occurs during tubular regeneration and proliferation [3]. These examples are consistent with the hypothesis that during tissue regeneration, a cascade of developmental gene pathways may be reactivated. Here we examine the expression

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pattern of *Pax-2*, a transcription factor that is transiently expressed during nephrogenesis and that may be part of the genetic cascade leading to kidney regeneration in adulthood after kidney damage. If so, we speculated that *Pax-2* would show a biphasic expression pattern during kidney development and disease. Currently, the role of *Pax-2* during epitheliogenesis is not understood. It is known, however, that *Pax-2* is important for the development of the pronephric, mesonephric, and metanephric systems [11, 12]. In mice, it is expressed in the ureteric bud at embryonic day 10.5 (E10.5), in the condensed mesenchyme at E12, and in comma- and s-shaped bodies at E12 through E13. During epithelial differentiation and maturation, *Pax-2* expression is down-regulated and is not detected in mature glomeruli and proximal tubuli [11, 13, 14]. *Pax-2* remains detectable in collecting tubular cells, which are cell derivatives of the embryonic ureter. The correct up-regulation and down-regulation of *Pax-2* expression is extremely important for proper nephrogenesis [15, 16]. During regeneration of the adult kidney, *Pax-2* may play a critical role by influencing proliferation.

The administration of folic acid to mice can lead to a transient ATN. An intraperitoneal folic acid injection at a dose of 200 to 250 mg/kg body wt produces tubular obstruction by intratubular crystallization, resulting in transient renal failure peaking at 6 to 24 hours after injection. As a consequence, the regeneration process initiates DNA and protein synthesis [4, 17, 18]. Among the nephron structures affected by toxins such as folic acid are the proximal tubular cells [19]. Therefore, this model is suitable to study changes of gene expression patterns during tubular regeneration.

In order to test the hypothesis that developmental genes are re-expressed during regeneration, we examined the expression pattern of the developmental gene *Pax-2* after experimentally induced ATN in adult mice. Our data demonstrate that *Pax-2* is transiently re-expressed in proximal tubular cells, which may be of functional significance during the regeneration of damaged tubular epithelium.

METHODS

Induction of ATN

Thirty male CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA, USA) weighing 31 ± 4 g received 250 mg/kg body wt folic acid dissolved in 0.5 ml 300 mM sodium bicarbonate solution by intraperitoneal injection. Thirty mice were used as negative controls and received 0.5 ml 300 mM bicarbonate solution only. At each time point (after 0, 3, 6, 24, and 72 hr) and for each group (folic acid or bicarbonate injection), six mice were sacrificed. Kidneys were quickly removed, and one was shock frozen in liquid nitrogen, whereas the other was processed for histological evaluation.

Determination of functional damage and histological studies

Blood was obtained by aspiration from the left ventricle to measure serum levels of creatinine and blood urea nitrogen (BUN) using a Kodak Ektachem 500 Analyzer (Kodak, Rochester, NY, USA).

For evaluation by light microscopy, kidneys were fixed in 4% paraformaline. Seven micrometer thick sections were cut on a microtome (Rotationsmikrotom 3455 Leitz; Leica, Bensheim, Germany) and periodic acid-Schiff (PAS) staining was carried out to document histological damage.

Immunohistochemistry

For indirect immunofluorescence studies, 3 to 4 mm thick frozen mouse kidney sections were covered with tissue freezing medium (Leica) and frozen on dry ice. Cryostat sections (Kryotom Jung CM 3000; Leica) were cut at 7 μm thickness, collected on gelatinized slides, and air dried for 60 minutes. Tissue sections were incubated with a Pax-2 polyclonal antibody as described [13, 20] using a TRITC-conjugated secondary antibody for fluorescence detection. Tissue sections were also incubated with a monoclonal anti-vimentin antibody (Dianova, Heidelberg, Germany); a Cy2 (Dianova)-conjugated secondary antibody was used for fluorescence detection.

Western blotting

For Western blotting analysis, thin cortical sections (approximately 1 mm thick) were homogenized in a glass homogenizer with boiling extraction buffer (62.5 mM Tris, pH 6.8, 1% sodium dodecyl sulfate, 10% glycerol, 5% β-mercaptoethanole). After the determination of protein concentrations using the Bio-Rad Dc Protein-Assay (Bio-Rad, Hercules, CA, USA), concentrations were standardized. Western blotting was performed as described by Harlow and Lane [21]. Negative (liver) and positive (embryonic murine kidney of E15) controls were used in parallel. Sixty micrograms protein per lane were loaded, except for the positive control (10 μg).

In situ hybridization

Single-stranded RNA probes were generated by *in vitro* transcription of a Bam HI-Eco RI fragment from the cDNA clone C31 A of murine *Pax-2* (500 bp fragment), generously provided by G. Dressler and P. Gruss. *In vitro* transcription was carried out using a Trans-Probe-T kit (Pharmacia, Freiburg, Germany) and digoxygenin-labeled uridine triphosphate (Boehringer, Mannheim, Germany). The vector [pBluescript KS (+); Stratagene, Heidelberg, Germany] was cut with BamHI and transcribed with T3-RNA polymerase to yield antisense probe. To generate a sense probe, the same plasmid was cut with EcoR1 followed by transcription with T7 RNA

polymerase. After deparaffinization, kidney sections were digested with 20 μg/ml proteinase K (Boehringer) in phosphate-buffered saline for 16 minutes. Sections were postfixed for five minutes in 4% formaldehyde and acetylated (0.25% acetic anhydride in 0.1 M triethanolamine for 10 minutes). *In situ* hybridization with digoxygenin mRNA, antidigoxygenin antibody incubation, and alkaline phosphatase reaction were done according to the manufacturer's guidelines (Boehringer).

Reverse transcription-polymerase chain reaction

Pax-2 mRNA expression after folic acid-induced ATN in mice was analyzed via reverse transcription-polymerase chain reaction (RT-PCR) as previously described [22]. In brief, RNA was isolated from kidneys using a phenol/guanidine isothiocyanate reagent (TRI-Reagent; Molecular Research Center, Cincinnati, OH, USA). Contaminating genomic DNA was removed with RNase free DNase (Promega Biotech, Madison, WI, USA). Oligo dT-primed reverse transcription was performed at 42°C for one hour after denaturation of the RNA at 65°C for five minutes with a modified MMLV-reverse transcriptase (Superscript; Life Technologies GmbH, Eggenstein, Germany). The cDNA concentration was adjusted such that aliquots yielded constant amounts of PCR amplification products of adenine nucleotide carrier mRNA, a mitochondrial transport molecule with stable expression after ATN [23]. PCR was performed using oligonucleotide primers selected from the sequence of the mouse adenine nucleotide carrier (Genbank accession: x74510): sense 5′-GCTGGACTTTGC TAGGAC-3′ (492 to 509 bp), antisense 5′-AAAGCAC CACCCATGCCTC-3′ (932 to 950 bp), which yielded a 458 bp product. Following denaturation for 3.5 minutes at 95°C, samples were cycled 30 times for one minute at 54°C and for 0.5 minute at 72°C; the final extension was performed using serial tenfold dilutions of cDNA from each time point. Oligonucleotide primers were selected from the published sequences [11]: sense 5′-GAGATG TGTCTGAGGGCTCTG-3′ (1004 to 1024 bp), antisense 5′-GTAACTAGTGGCGGTCATAGGC-3′ (1555 to 1576 bp), yielding a 572 bp product. As a positive control, a known copy number of plasmid DNA containing the full-length mouse *Pax-2* cDNA in serial dilutions and E17.5 mouse kidney cDNA were used. The cDNA reaction mix from RT minus reactions was also included in each PCR run; these samples were consistently negative. To assess product abundance, 12 μl of each radiolabeled PCR reaction were separated by electrophoresis on a 5% acrylamide gel. The intensity of product bands was quantitated using the Imagequant software on a Storm 640 Molecular Imager (Molecular Dynamics, Krefeld, Germany).

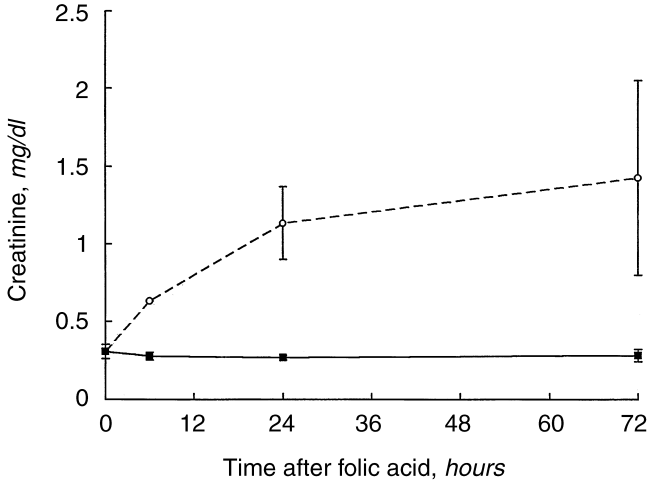


Fig. 1. Time course of creatinine values in the early phase of acute tubular necrosis. Blood samples were drawn from mice until 72 hours after injection of folic acid (250 mg/kg body wt) versus control solution (300 mM bicarbonate). Bars represent mean serum values ($N = 2$ for every time point) ± SEM. In the control group (■), there was no alteration of the serum creatinine levels after injection, whereas in the experiment group (○) there was a fourfold to fivefold increase in creatinine levels after folic acid injection up to 72 hours.

RESULTS

Determination of functional and histological damage

The induction of renal damage by intraperitoneal folic acid injection was verified by the observed rise of serum creatinine and BUN levels as coarse indicators of renal function (the time course of creatinine values is shown in Fig. 1). Both creatinine and BUN increased approximately fourfold to fivefold during the early phase of ATN (24 to 72 hr after folic acid injection). No alteration of creatinine and BUN levels was observed up to 72 hours after control injections with bicarbonate solution. The animals in the folic acid group started to show signs of disease such as fatigue, reduced alertness, and bristling of the coat at 12 to 24 hours after the folic acid injection. These signs were absent in the control injected group. All animals survived up to the planned time point of sacrifice.

Periodic acid-Schiff staining of kidney sections after the chemically induced damage showed alterations in kidney morphology consistent with ATN, such as disrupted brush borders and flattening of epithelia (Fig. 2b). In summary, an injection of folic acid resulted in the expected functional and morphological alterations of ATN with transient impairment of kidney function. No changes were observed in the vehicle-injected control mice.

Indirect immunofluorescence studies

Pax-2 was found to be expressed in cells of the comma- and s-shaped bodies, which are epithelial precursors of

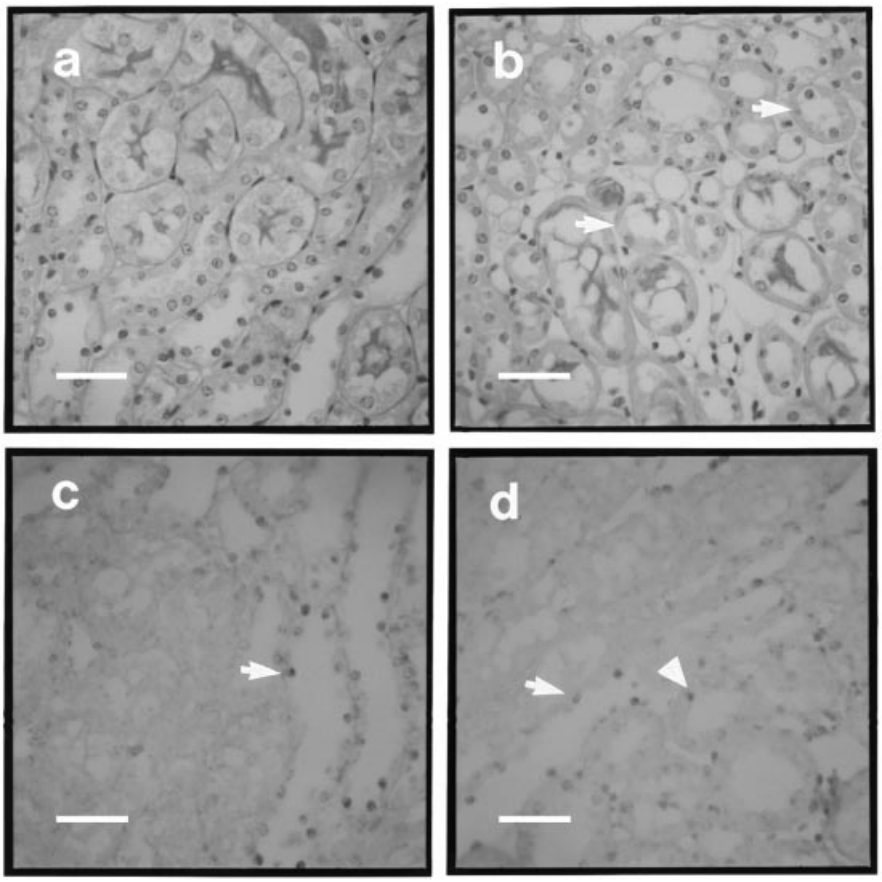


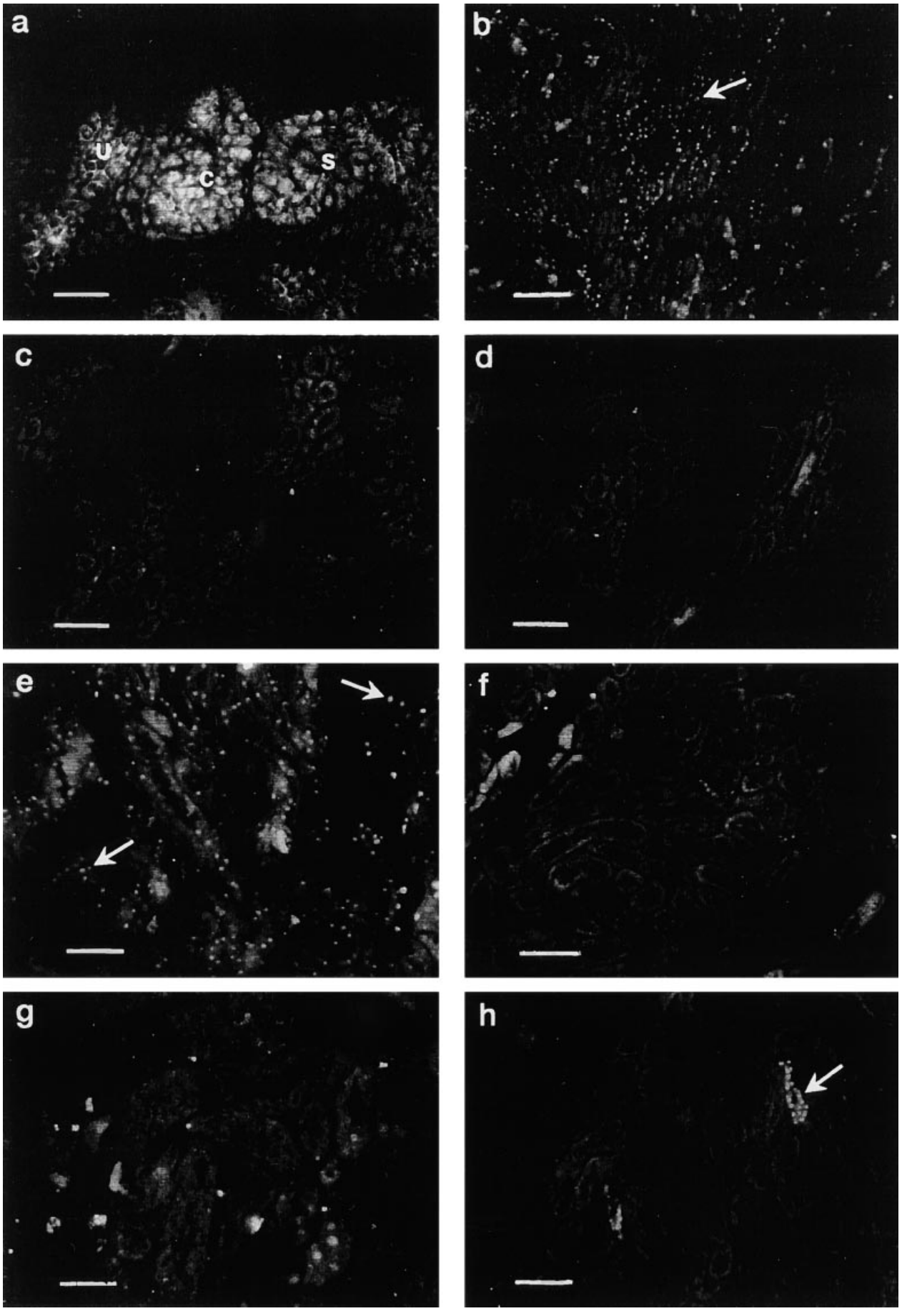
Fig. 2. Periodic acid-Schiff (PAS) staining and *in situ* hybridization for *Pax-2* mRNA in murine kidney sections. (*a* and *b*) Histological damage documented by PAS staining of kidney sections 24 hours after folic acid versus control injection (bars, 16 μm). (a) Kidney sections 24 hours after sodium bicarbonate administration with regular glomeruli and proximal tubules. (b) Straight segments of proximal tubules show a disrupted brush border and a flattening of epithelia 24 hours after folic acid administration, indicating tubular damage (arrows). (*c* and *d*) *In situ* hybridization for *Pax-2* mRNA in kidneys undergoing ATN versus control kidneys (bars, 25 μm). (c) A kidney section 24 hours after sodium bicarbonate administration with a positive nuclear signal only in collecting ducts (arrow). (d) A kidney section 24 hours after folic acid administration with positive signals in nuclei of damaged tubules (arrowhead) in addition to collecting ducts (arrow).

the developing proximal tubules. In our study, we used the embryonic mouse tissue (E19) as a positive control (Fig. 3a), where differentiation and epitheliogenesis is still present. The staining pattern is exclusively nuclear, showing sharp boundaries between positive and negative areas.

ATN produced by the intraperitoneal injection of folic acid into mice leads to an altered expression pattern of *Pax-2* protein in the kidney. In the healthy adult kidney, *Pax-2* could be only detected in collecting duct nuclei, with the highest expression in the papilla (Fig. 3b). Cells of the proximal tubules, or glomeruli, were devoid of *Pax-2* protein expression in the cortex (Fig. 3c).

No change of *Pax-2* expression was observed at three hours after folic acid administration (Fig. 3d). In contrast, at 24 hours, a marked re-expression of *Pax-2* protein was observed in proximal tubular cell nuclei (Figs. 3e and 4). This result was obtained for all of the animals of the group ($N = 6$). The positive cells could be identified as belonging to regenerating proximal tubules due to the high-prismatic epithelium and remnants of the damaged brush border detected in the lumen of the tubules. The expression of *Pax-2* in proximal cell nuclei was found to decrease by 72 hours after an injection of folic acid. At this point, *Pax-2* was barely detectable in

Fig. 3. *Pax-2* immunofluorescence of cryosections from murine kidneys undergoing acute tubular necrosis (ATN). A murine neonatal kidney (E19) stained with anti–*Pax-2* antibodies served as a positive control for *Pax-2* expression. (*a*) In the neonatal kidney (E19), sharp boundaries exist between positively and negatively staining substructures. Nuclear localization of *Pax-2* protein can be observed in cells of the ureter (u), s-shaped bodies (s), and comma-shaped bodies (c), whereas the surrounding tissue, the uninduced mesenchyme, is devoid of *Pax-2* protein (bar, 16 μm). (*b* and *c*) Cryosections of a healthy adult mouse kidney stained with anti–*Pax-2* antibodies. (b) In the papillary region, *Pax-2* can only be detected in the nuclei of the collecting duct cells (arrows; bar, 50 μm). (c) No signal can be detected in the tubular cells of the cortex of a healthy murine kidney (bar, 50 μm). (*d*) At three hours after an injection of folic acid, there is no change for *Pax-2* staining as compared with the zero time point (bar, 50 μm). After six hours, there is still no change of the immunofluorescence pattern in the folic acid or the control group (data not shown). (*e*) A marked re-expression of *Pax-2* can be observed 24 hours after folic acid injection in proximal tubular cell nuclei (arrows), whereas no change is noted in other parts of the nephron (bar, 50 μm). (*f*) In controls, no re-expression of *Pax-2* can be observed after 24 hours (bar, 50 μm). (*g*) Seventy-two hours after folic acid administration, the *Pax-2* immunofluorescence was markedly decreased in proximal tubular cell nuclei (bar, 25 μm). The expression pattern resembles the one observed in the control group (*h*, arrow indicating a collecting tubule positive for *Pax-2;* bar, 50 μm).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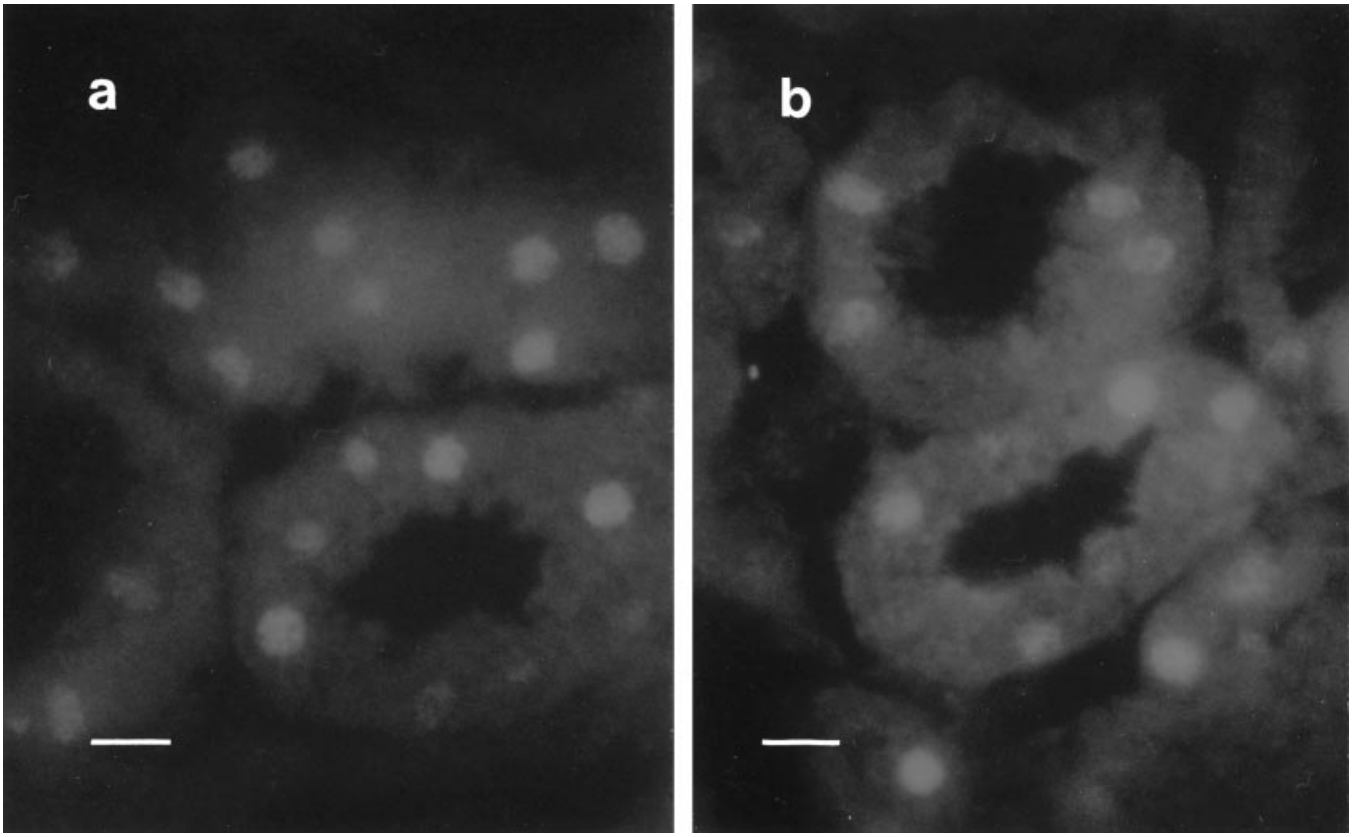

Fig. 4. Murine kidney cryosections of two different animals (*a* and *b*) obtained 24 hours after folic acid injection and stained for *Pax-2*. The nuclear expression pattern of *Pax-2* protein is detectable in proximal tubular epithelial cells (bars, 10 μm).

the proximal tubules, and expression was again restricted to collecting duct cells (Fig. 3g). In control bicarbonate-injected animals, no increase of *Pax-2* protein expression in proximal tubular cells was observed at any of the time points examined (Fig. 3f, 24 hr after control injection; Fig. 3h, 72 hr after control injection). *Pax-2* remained detectable exclusively in collecting duct cells in the control animals. Vimentin was detected and coexpressed with *Pax-2* in folic acid-injected animals 24 hours after injection (Fig. 5). No expression could be observed in control injected animals.

Analysis by Western blotting

To complement the observation of the increase of *Pax-2* immunofluorescence signal in proximal tubular cells after folic acid-induced ATN, we examined *Pax-2* protein concentration in kidney homogenates by Western blotting (Fig. 6). In E15 embryonic mouse kidney homogenates, which served as positive controls, three bands could be detected with molecular weights of 42, 46, and 48 kDa, respectively. The intensity was strongest in the 46 kDa band, which corresponds to the *Pax-2*b isoform, which is most abundant *in vivo* [13]. In the negative control, a murine liver homogenate, no specific bands could be observed. To analyze the effect of folic acid-induced ATN, freshly prepared extracts from cortical kidney sections were analyzed with anti–*Pax-2* antibodies. To minimize the possible contaminating effect of the basal expression of *Pax-2* protein in the normal papillary region (Fig. 3b), which had to be distinguished from newly synthesized *Pax-2* protein in the cortex, total cell extracts, including the nuclear proteins of thin cortical sections of murine kidneys, were examined. The control kidney cortex homogenate (time point 0 hr) showed a very weak 46 kDa band, which probably represented the *Pax-2* protein present in collecting ducts derived from cortical sections (as shown by immunofluorescence). Comparable to the increase of *Pax-2* protein in proximal tubules observed by immunofluorescence studies 24 hours after ATN, an increase of *Pax-2* protein levels was also detectable by Western blotting of cortical homogenates. The up-regulation of *Pax-2* protein expression was temporary. Seventy-two hours after the induction of ATN, the intensity of the 46 kDa *Pax-2* band declined. In comparison to the *Pax-2* band 24 hours after kidney damage, the bands of the control group (24 and 72 hr after bicarbonate injection) showed a much weaker intensity.

In situ hybridization

In healthy adult murine kidney, *Pax-2* mRNA is detectable exclusively in the nuclei of collecting duct cells

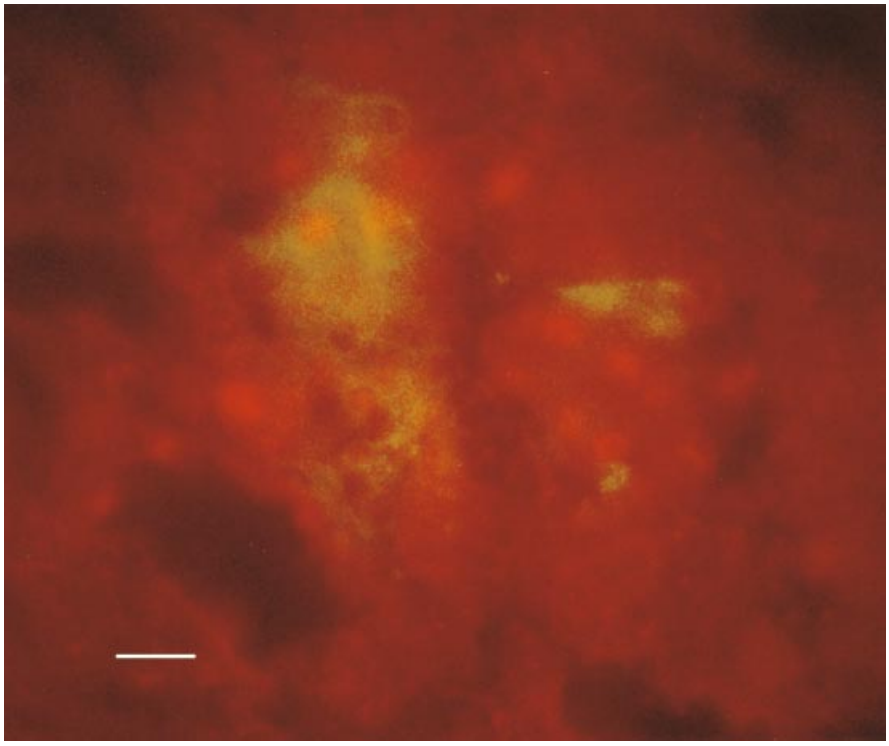

Fig. 5. Vimentin (green fluorescence) and *Pax-2* (red) coexpression in proximal tubules of a cortical kidney section 24 hours after folic acid-induced ATN (bar, 10 μm).

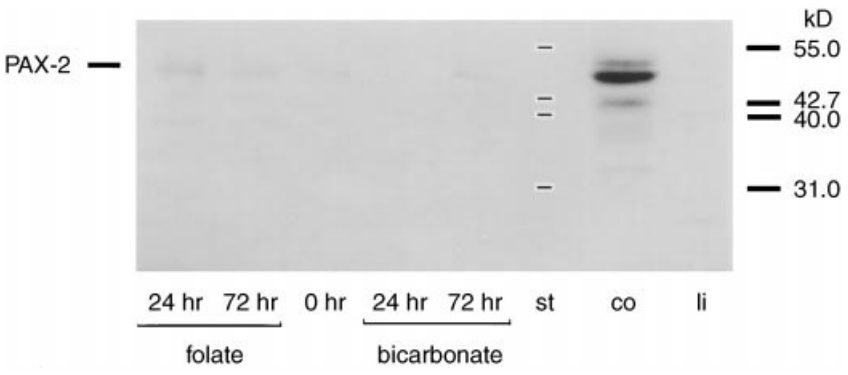

Fig. 6. A Western blot for *Pax-2* with protein extracts from cortical kidney sections obtained at different time points after injection of folic acid versus control solution (bicarbonate). In the positive control (co, E15), two bands can be detected with a molecular weight of 46 and 48 kDa, respectively. In the negative control (li, adult murine liver), no specific bands are observed after incubation with anti–*Pax-2* antibodies. The lanes termed "folate" were loaded with homogenates of kidneys from mice sacrificed at the indicated time points after folic acid injection. In the murine kidney homogenate at time point of 0 hours, a band of 46 kDa weight is seen. Twenty-four hours after folic acid injection, the intensity of this band shows an increase, indicating increased *Pax-2*–protein concentration. The *Pax-2* band has declined in kidney homogenates obtained 72 hours after folic acid injection. The intensity of the *Pax-2* bands in the control injected group (after 24 and 72 hr) is much lower than the intensity of the band 24 hours after folic acid injection. A 60 μg protein was loaded per lane, except of the positive control, where 10 μg were loaded.

and to a smaller extent in the cells of the distal tubules. No *Pax-2*–positive cells could be detected in proximal tubules 24 hours after bicarbonate injection (Fig. 2c). In contrast, 6 and 24 hours after folic acid injection, the proximal tubular epithelia of the outer medulla and inner cortex showed a spotty nuclear signal for *Pax-2* that was most pronounced in damaged epithelia (Fig. 2d). Seventy-two hours after folic acid injection, this spotty signal had disappeared. The fact that *Pax-2* mRNA was detectable only in the nuclei but not in the cytoplasm of damaged cells could be due to a short half-life of the mature *Pax-2* mRNA. A sufficient amount of *Pax-2* mRNA to be detectable by *in situ* hybridization would then be expected to be present only at the place of its origin, in the nucleus.

Reverse transcriptase-polymerase chain reaction studies

A specific band of the expected size for *Pax-2* could be detected in quantitative RT-PCR experiments of RNA from cortical mouse kidneys in control as well as in

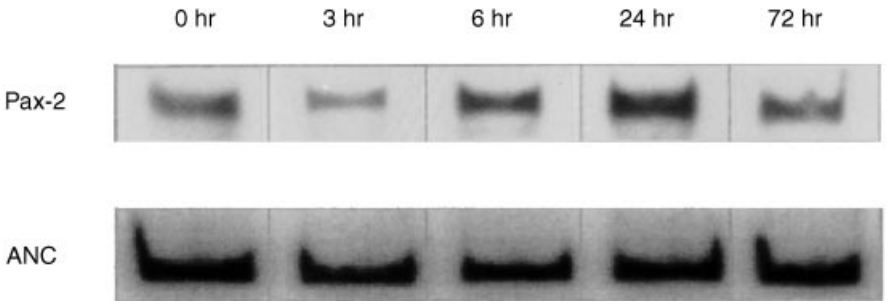

Fig. 7. Reverse transcription-polymerase chain reaction (RT-PCR) for *Pax-2* from RNA of cortical kidney sections after folic acid induced acute tubular necrosis (ATN). As a control the levels of adenine nuclear carrier (ANC) mRNA was examined, which is not differentially expressed after proximal tubular damage. *Pax-2* mRNA shows an increase after folic acid injection with a maximum at 24 hours after induction of ATN.

experimental animals. In the folic acid-injected group, there was a twofold to threefold increase in the intensity of the signal 24 hours after injection compared with the 0-hour value (Fig. 7) or the control-injected animals (data not shown). No change was observed in mRNA for adenine nuclear carrier (ANC) as the control housekeeping gene was not affected by ATN [23]. Thus, the increase in *Pax-2* protein, as determined semiquantitatively by Western blot analysis, is also accompanied by an increase of *Pax-2* mRNA.

DISCUSSION

Regeneration processes are characterized by proliferation of dedifferentiated cells and later redifferentiation of the daughter cells into the required cell phenotype. A similar sequence of events can also be observed during embryogenesis. Therefore, it was postulated that regeneration processes may recapitulate parts of the genetic program, which is evident during organogenesis in order to reestablish proper tissue function after damage [2, 3]. One way to test this hypothesis is to use a standard model of acute renal failure in mice and to examine the expression patterns of developmental control genes during regeneration in those injured adult animals.

Pax-2 is known to play a crucial role during early metanephric kidney development. We observed a close timely correlation between the impairment of kidney function and the re-expression of *Pax-2* in regenerating tubular epithelium, which may indicate a functional link. After kidney injury, *Pax-2* was locally restricted and re-expressed in the regenerating proximal tubules. This expression was limited to a time interval of 6 to 72 hours, peaking 24 hours after the folic acid injection. Similar to its transient expression pattern during development [13, 14], the expression in proximal tubular cells declined after reconstitution of the tubuli, beginning 72 hours after the induction of ATN. This transient re-expression of *Pax-2* in hyperproliferative proximal tubular epithelia after ATN is paralleled by the findings of Winyard et al, who showed deregulated expression of *Pax-2* in cystic and hyperproliferative dysplastic epithelia in human kidney malformations [24]. In our study, *Pax-2* was coexpressed with vimentin, a mesenchymal marker. Witzgall et al had shown that proliferating cell nuclear antigen (PCNA) and vimentin appear to be coexpressed in proliferating and regenerating proximal tubulus epithelia [19]. We suggest that the regulated transient expression of *Pax-2* after ATN correlates with the regulated and timely-restricted hyperproliferative state of the proximal tubule. On the other hand, deregulated *Pax-2* expression may lead to tubular malformations caused by uncontrolled hyperproliferation, both in human disease [24] and during murine embryonic development [16]. High expression levels of *Pax-2* have also been shown to be present in malignancies such as Wilms' tumor [13] and renal cell carcinoma [25]. Furthermore, several members of the *Pax* family, including *Pax-2*, have oncogenic potential [26].

It remains to be shown which factors induce the transient up-regulation of *Pax-2* after folic acid-induced ATN and how *Pax-2* expression is down-regulated after its transient expression during ATN. Recently, it was demonstrated in an *in vitro* organ culture system that *Pax-2* up-regulation was detected already four hours after the application of lithium [27]. In the adult kidney, lithium uses the promiscuous sodium transporter to exert its function. During acute tubular necrosis, there is extensive backleak of filtrate across the damaged tubular epithelium, leading to a high intracellular sodium concentration. Thus, salt overload of the damaged epithelial cell might lead to the activation of *Pax-2.* Moreover, *Pax-2*–mediated regeneration might be influenced via STAT-3, which recently was shown to be responsible for *HGF*-mediated tubulogenesis in cell culture [28]. Other possible genes that might influence tubulogenesis include *Wnt-4*, *Lim-1*, and *BF-2* [29].

Thus far, target genes for *Pax-2* remain unknown, although recently two genomic clones were identified that contain *Pax-2* binding sites and therefore can be used as sequence tags to identify potential target loci [30]. The identification of upstream and downstream events that are involved in the transient expression of *Pax-2* during ATN will provide more insights in the mechanisms that are important for and possibly link kidney development and regeneration.

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APPENDIX

Abbreviations used in this article are: ANC, adenine nuclear carrier; ATN, acute tubular necrosis; BUN, blood urea nitrogen; E, embryonic day; RT-PCR, reverse transcription-polymerase chain reaction.

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