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Compensatory renal hypertrophy is mediated by a cell cycle-dependent mechanism

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Compensatory renal hypertrophy is mediated by a cell cycle-dependent mechanism.

Background. Two mechanisms exist for inducing renal proximal tubule hypertrophy. One is characterized by regulation of the G_1 cell cycle kinase (cell cycle-dependent mechanism), while the other mechanism involves an imbalance between rates of protein synthesis and degradation, and occurs independently of cell cycle kinase regulation (cell cycle-independent mechanism). The present studies examined whether the compensatory proximal tubule growth following uninephrectomy is mediated by the cell cycle-dependent or -independent mechanism.

Methods. Studies were done in both rats and C57Bl6 mice on tissue harvested from sham-operated or uninephrectomized animals. The magnitude of BrdU incorporation was used as the hyperplasia marker, while the proximal tubule protein: DNA ratio was used as the hypertrophy marker. Cdk4/cyclin D and cdk2/cyclin E kinase activities were assayed on renal cortex (rat studies) or isolated proximal tubules (mouse studies) using an in vitro kinase assay.

Results. In both rats and mice, compensatory proximal tubule growth was hypertrophic, not hyperplastic, evidenced by an increase in the protein:DNA ratio without a change in BrdU incorporation. In mice, cdk4/cyclin D kinase activity progressively increased between days 4 and 7, while cdk2/cyclin E kinase activity was decreased at both 4 and 7 days. In rats the development of hypertrophy was associated with an increase in cdk4/cyclin D kinase at days 4, 7, and 10, and an increase in cdk2/cyclin E kinase activity at days 2, 4, and 7. Roscovitine, a cdk2/cyclin E kinase inhibitor, inhibited cdk2/cyclin E kinase activity in both sham and nephrectomized rats; however, it did not prevent the development of proximal tubule hypertrophy.

Conclusions. Uninephrectomy-induced compensatory proximal tubule growth is a hypertrophic form of growth that is mediated by a cell cycle-dependent mechanism.

Kidney size increases in a number of physiological and pathological situations, including uninephrectomy, diabetes mellitus, and chronic electrolyte imbalances. The majority of the growth occurs in the cortex, particularly in the proximal convoluted tubules, and appears to be of a hypertrophic, rather than hyperplastic nature. The mechanism(s) responsible for initiating the hypertrophic growth response are not well understood.

We have characterized two mechanisms that can cause renal epithelial cells to hypertrophy [1–7]. One of these, referred to as a cell cycle-dependent hypertrophy mechanism, involves the coordinated effects of a mitogen and antiproliferative agent. In this model, a mitogenic stimulus "moves" quiescent renal epithelial cells into the G_1 phase of the cell cycle, where they initiate events typical of early G_1, including activation of cdk4(6)/cyclin D kinase and stimulation of protein synthesis. The antiproliferative agent, transforming growth factor-β (TGF-β) in the systems we have used, arrests cell cycle progression prior to the restriction point, preventing activation of cdk2/cyclin E kinase. Failure to activate cdk2/cyclin E kinase results in the retinoblastoma protein, pRB, remaining activated, E2F-induced transcription being blocked, and failure of the cells to cross the restriction point and move into S phase. However, having undergone the increase in physical growth associated with the early G_1 phase, the arrested cells are now "trapped" in a physically enlarged or hypertrophied state.

The second mechanism involves an imbalance between rates of protein synthesis and degradation. This form of hypertrophy is induced by exposing cells to compounds, such as NH_4Cl, that alkalinize acidic intracellular vesicles, such as lysosomes. Increases in ambient NH_4Cl concentration in media have no effect on rates of protein synthesis, yet total cellular protein content increases, suggesting that rates of protein degradation have declined, leading to an "accumulation" of cellular protein and the development of an enlarged cell. This scenario is supported by studies showing that alkalinization of acidic intravesicular compartments inhibits the activity of lysosomal enzymes involved in protein degradation [8, 9]. A characteristic of this form of hypertrophy is that it occurs without regulation or involvement of cells cycle processes. Thus, the development of hypertrophy is not

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associated with cdk4/6:cyclin D kinase activation, and occurs in the absence of the activated form of pRB. This form of hypertrophy is referred to as being cell cycle-independent.

The purpose of the present studies was to determine if the renal hypertrophy associated with the loss of renal mass (uninephrectomy) is mediated by a cell cycle-dependent or -independent mechanism. The studies show in both rats and mice that proximal tubule growth following uninephrectomy is a hypertrophic, and not a hyperplastic growth form, and that the development of hypertrophy is associated with activation of cdk4/6:cyclin D, but not physiologically significant regulation of cdk2:cyclin E kinase. This pattern of cell cycle kinase activity suggests that uninephrectomy-induced proximal tubule hypertrophy is mediated by a cell cycle-dependent process.

METHODS

Compensatory renal growth model

Compensatory renal growth was induced by uninephrectomy in both male rats (Sprague-Dawley rats weighing 200 to 250 g) and male mice (C57 Black 6 mice, 6- to 8-weeks-old). All animals were allowed free access to standard rodent chow and water throughout the study. Animals were randomly divided into two groups: sham-operated or left nephrectomy. Sham surgery and uninephrectomies were performed as previously described by us [10].

In some studies, the cdk2/cyclin E kinase inhibitor roscovitine was used [11–13]. Roscovitine [3.5 mg/kg body weight (BW)] or vehicle [dimethyl sulfoxide (DMSO) and saline] was administered intraperitoneally to sham and uninephrectomized rats 24 hours prior to surgery, and then daily until sacrifice four days post-surgery.

DNA synthesis

Rates of DNA synthesis were assayed using 5-bromo-2-deoxyuridine (BrdU) incorporation, as previously reported by us [7]. Two hours prior to sacrifice, animals received a single intraperitoneal injection of 100 μg BrdU/g BW. When the kidneys were harvested (see later in this section), a central transverse section of kidney was placed in 10% formaldehyde overnight and then transferred to phosphate-buffered saline (PBS) until being paraffin embedded. A 3 to 4 μm slice was mounted on a lysine coated glass slide. Anti-BrdU antibodies were used to quantitate BrdU incorporation into DNA, as per kit protocol (Oncogene Research Products, Cambridge, MA, USA). Hematoxylin was used as the counterstain. Data are reported as the average number of BrdU positive cells in the outer cortex in five high power fields. Control studies, done in the absence of the anti-BrdU antibody (primary antibody), demonstrated the absence of non-specific staining.

Isolation of intact proximal tubules

Two different approaches were used to isolate proximal tubules. A Percoll gradient was used to isolate proximal tubules that would be used for measuring the protein:DNA content ratio, while a sieving approach was used to measure G_1 kinase [1]. In pilot studies it was found that the Percoll gradient inactivates the kinases, and thus, this approach to tubule isolation cannot be used for these studies. Both protocols are presented in the next paragraphs.

Isolation of proximal tubules by Percoll gradient and measuring the protein and DNA ratio

Rats or mice were anesthetized, and the right kidney removed through an abdominal incision and immediately placed in ice cold PBS. The superficial cortex was removed, sliced with a Stadie-Riggs tissue slicer, and put into ice cold PBS containing the following protease inhibitors: 2 μg/mL apotinin, 2 μg/mL pepstatin A, 5 μg/mL leupeptin, and 0.1 mg/mL phenylmethylsulfonyl fluoride (PMSF). The cortical slices were washed three times with Prep-Media (50:50 mixture of low glucose DMEM: Ham's F12; Gibco BRL, Gaithersburg, MD, USA), suspended in 10 mL of 95% O_2/5% CO_2 pre-equilibrated Prep-Media containing 1.8 mg/mL collagenase B (Boehringer Manneheim, Manneheim, Germany), and incubated in a 37°C shaking water bath for 10 minutes. After collagenase digestion, 10 mL of ice cold Prep-Media was added to the tissue suspension to stop the collagenase digestion reaction. The tissue suspension was then kept on ice continuously.

The digested tissue was filtered through a double layer of gauze (USP Type II gauze), the filtrate centrifuged at 800 rpm for 30 seconds, and washed twice with PBS. After the final wash, the pellet was resuspended in 6 mL prebubbled (95% O_2/5% CO_2), cold, 45% Percoll (3 mol/L NaCl, 154 mmol/L KCl, 200 mmol/L KH_2PO_4, 155 mmol/L $MgSO_4$, 110 mmol/L $CaCl_2$), and centrifuged at 14,000 rpm for 40 minutes at 4°C. The separated bottom band was carefully withdrawn using a Pasteur pipette, placed in 10 mL PBS, centrifuged at 800 rpm for 40 seconds at 4°C, the supernatant discarded, and the tubules rewashed. After the final washing, the pellet was resuspended in 1 mL hypotonic lysis buffer (50 mmol/L NaH_2PO_4, pH 7.4) and sonicated on ice for 5 seconds $\times 4$. Of the resulting suspension, 0.65 mL was diluted with the same volume of hypotonic buffer containing 2 mmol/L EDTA, and used to measure DNA content. The remaining aliquot (0.35 mL) was used to measure protein content. The protein and DNA concentrations of the proximal tubule lysate were measured by Lowry

(protein) and the fluorescent compound Hoechst H33258 (DNA), respectively, as previously reported by us [7].

Isolation of proximal tubules by sieving

Proximal tubules also were collected using a sieving technique [14, 15]. The collagenase-digested tissue described above was first passed through an 80-mesh size mini-sieve (Fisher Scientific, Pittsburgh, PA, USA). The flow through containing the proximal tubules was then passed through a 170-mesh size sieve. The proximal tubules, which now remained on the sieve filter, were collected and suspended in 20 mL Prep-Media, and the tubules pelleted by gravity sedimentation ×5 minutes. To insure removal of any remaining cellular debris, the sedimentation process was repeated ×2 after resuspending the tubules in ice cold PBS containing the protease inhibitors described earlier. The final sedimented pellet was kept on ice until used.

G_1 kinase activity

cdk2/cyclin E kinase activity. cdk2/cyclin E kinase activity was measured on whole cortex in the rat studies and on isolated proximal tubules in the mouse studies using a protocol similar to that which we have previously published [5, 7]. For the rat studies, outer cortex was homogenized in PBS containing protease inhibitors (2 μg/mL apotinin, 2 μg/mL pepstatin A, 5 μg/mL leupeptin, and 0.1 mg/mL PMSF) and centrifuged at 10,000 rpm for five minutes at 4°C. For the mouse studies, isolated proximal tubules were obtained by the sieving method described above. The homogenized cortical pellet (rat studies) or isolated proximal tubules (mouse studies) was resuspended in 1000 μL lysis buffer with protease inhibitors [150 mmol/L NaCl, 50 mmol/L Tris-HCl pH 7.4, 5 mmol/L EDTA, 0.5% Na-deoxycholate (DOC), 1% NP-40, 1 mmol/L Na_3VO_4, 1 mmol/L NaF, 1 mmol/L dithiothreitol (DTT), 20 μg/mL apotinin, 10 μg/mL pepstatin A, 10 μg/mL leupeptin, and 0.1 mg/mL PMSF] and rotated for 60 minutes at 4°C. After centrifugation at 10,000 rpm for 20 minutes at 4°C, the supernatant was used to measure the protein concentration of the resulting lysate, using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Cyclin E was immunoprecipitated by adding 1 μg rabbit anti-cyclin E antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) to 500 μg of cortical or tubular protein. The immunoprecipitation reaction was carried out overnight at 4°C with gentle rotation. The next day 100 μL protein-G agarose beads were added, and the sample incubated at 4°C with gentle rotation for one hour. The beads were collected by centrifugation at 10,000 rpm for 30 seconds at 4°C, the supernatant discarded, and the beads washed four times with 1 mL lysis buffer, followed by three additional washings with 1 mL kinase reaction buffer that did not contain either cold or hot adenosine 5′-triphosphate (ATP) or kinase substrate (see below). After the last wash, the bead pellet was resuspended in 30 μL of kinase reaction buffer containing ATP and kinase substrate [50 mmol/L Tris-HCl, pH 7.4, 10 mmol/L $MgCl_2$, 1 mmol/L DTT, 70 mmol/L NaCl, 0.1 mmol/L ATP, 0.2 μg/μL histone H1, and 0.2 μCi/μL [γ-^{32}P]ATP (6000 Ci/mmol)]. The reaction was carried out by gently shaking the sample in a 30°C water bath × 30 minutes. The kinase reaction was stopped by the addition of 7.5 μL Laemmli sample buffer. The samples were then boiled for three minutes and size separated by SDS-PAGE on a 12% gel. The gel was stained with Coomassie blue stain to confirm equal amounts of kinase substrate in each sample, destained, and dried. Phosphorylated substrate was visualized by autoradiography and quantitated by densitometry.

cdk4/cyclin D kinase activity. cdk4/cyclin D kinase activity was measured using the same protocol described above with the following exceptions, as previously reported by us [5, 7]. The lysis buffer contained 150 mmol/L NaCl, 1 mmol/L EDTA, 2.5 mmol/L egtazic acid (EGTA), 10% glycerol, 0.1% Tween 20, 1 mmol/L DTT, 0.1 mmol/L Na_3VO_4, 1 mmol/L NaF, 10 mmol/L β-glycerophosphate, 20 μg/mL aprotinin, 10 μg/mL pepstatin A, 10 μg/mL leupeptin, and 0.1 mg/mL PMSF. The immunoprecipitating antibody was a rabbit anti-cdk4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The cdk4 kinase reaction buffer contained 10 mmol/L $MgCl_2$, 50 mmol/L HEPES pH 7.5, 2.5 mmol/L EGTA, 10 mmol/L β-glycerophosphate, 0.1 mmol/L Na_3VO_4, 1 mmol/L NaF, 1 mmol/L DTT, 20 μmol/L ATP, 0.2 μCi/μL [γ-^{32}P] ATP (6000 Ci/mmol), and 0.01 μg/μL GST-pRB [pRB amino acids 769-921 fused to glutathione S-transferase (GST); Santa Cruz Biotechnology].

Statistics

Statistical significance was determined by the appropriate *t* test. Differences between means were considered significant if $P < 0.05$ or less.

RESULTS

Compensatory renal growth in the rat following uninephrectomy

As has been shown by other laboratories, following a left uninephrectomy there is a progressive increase in right kidney weight (Fig. 1). The first studies done to determine the type of growth responsible for this kidney enlargement were to examine right kidney proximal tubule BrdU incorporation and the protein:DNA ratio of isolated proximal tubules harvested from the right kidney during the growth period (Table 1 and Fig. 2, respectively). As shown, there was no increase in BrdU incorporation at days 2, 4, and 14, but there was a progressive increase in the protein:DNA ratio. These data demon-

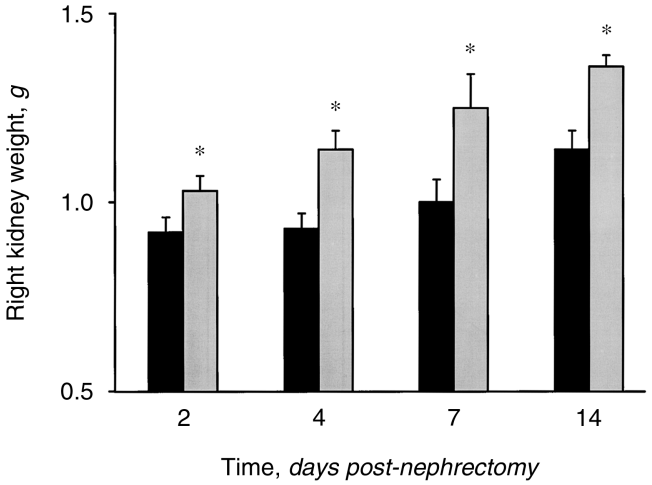

Fig. 1. Right kidney weight in rats post-uninephrectomy. A left nephrectomy (■) or sham surgery (■) was performed and the right kidney harvested and weighed as described in the **Methods** section. Number of sham/uninephrectomy pairs = 6 (2 days), 14 (4 days), 10 (7 days), and 7 (14 days). *$P < 0.05$ vs. sham-operated animals.

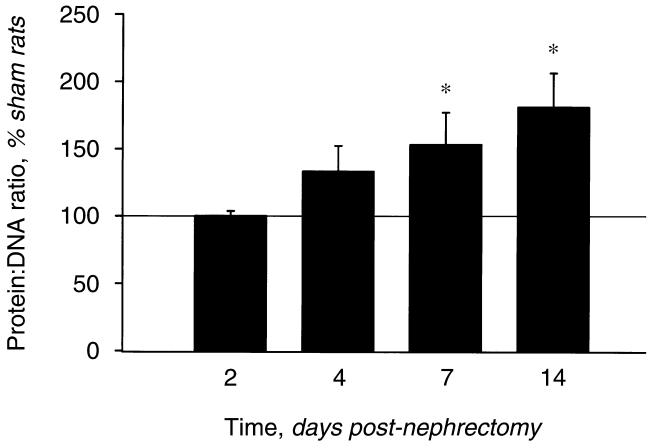

Fig. 2. Protein:DNA ratio in rat isolated proximal tubules post-uninephrectomy. Proximal tubules were isolated and the Protein:DNA ratio measured as described in the **Methods** section. Number of sham/uninephrectomy pairs = 5 (2, 4, and 7 days) and 8 (14 days). *$P < 0.05$ vs. sham-operated animals.

Table 1. Compensatory renal activity in the rat and mouse models after surgery

	BrdU incorporation[a]			
	2 Days	4 Days	7 Days	14 Days
Rat				
Sham	2.2 ± 0.3	2.3 ± 0.8	0.8 ± 0.3	4.9 ± 1.3
Uninephrectomy	5.3 ± 1.6	4.1 ± 1.6	4.3 ± 1.6	6.9 ± 1.8
Mice				
Sham		1.8 ± 0.5	1.2 ± 0.4	
Uninephrectomy		1.7 ± 0.4	2.0 ± 0.5	

[a] Days post-nephrectomy; average number of BrdU positive cells/high power field; N = 5–9/group; P = NS, except for the 7 day rat study (see text for explanation)

strate that compensatory renal growth is a hypertrophic, and not a hyperplastic growth process. There was, at 7 days post-nephrectomy, a significant increase in BrdU incorporation due to a smaller magnitude of incorporation in the sham-operated rats compared to the other time points. Since the magnitude of BrdU incorporation in the nephrectomized rats was similar to the other time points, we do not believe that this represents a proliferative response at 7 days.

G_1 kinase activity following uninephrectomy in the rat

Figure 3 profiles the time course of cdk4/cyclin D and cdk2/cyclin E kinase activity following uninephrectomy. cdk4/cyclin D kinase activity was increased at 4 days post-nephrectomy, and remained elevated but then decreased toward baseline at 7 and 14 days [day 2, 6% decrease (3/5), $N = 5$; day 4, 42% increase (3/4), $N = 4$; day 7, 14% increase (4/5), $N = 5$; day 14, 27% increase (3/5), $N = 5$.* cdk2/cyclin E kinase activity was increased at 2, 4, and 7 days, and slightly inhibited at 14 days [day 2, 74% increase (5/5), $N = 5$; day 4, 56% increase (4/4), $N = 4$; day 7, 66% increase (5/5), $N = 5$; day 14, 17% decrease (4/5), $N = 5$]. The observation that the increase in cdk4/cyclin D kinase activity correlates with the beginning of the increase in protein:DNA ratio suggests that movement into the G_1 phase of the cell cycle is needed to initiate the hypertrophic growth process. Although cdk2/cyclin E kinase activity also increased, there was no increase in BrdU incorporation (Table 1), suggesting that the increase in cdk2/cyclin E kinase activity was insufficient to inactivate pRB and move the cell into the S phase. By comparison, following a transient renal ischemic period, the hyperplastic growth response is marked by BrdU incorporation increasing from an average of 3 (sham) to >100 (renal ischemia) BrdU-positive cells/high powered field. This increase in BrdU incorporation was associated with a >700% increase in cdk2/cyclin E kinase activity (unpublished observations).

Compensatory renal growth following uninephrectomy in the mouse

To determine if there is a species differences in the renal growth pattern following uninephrectomy, studies were performed in C57Black 6 mice (Fig. 4). As shown, at both 4 and 7 days post-nephrectomy, there is an increase in right kidney weight and the protein:DNA ratio in isolated proximal tubules, but no change in BrdU incorporation (Table 1), demonstrating that the kidney

*The number in parentheses indicates the number of experiments in which the individual experimental result was in the same direction as the mean divided by the total number of experiments for that protocol.

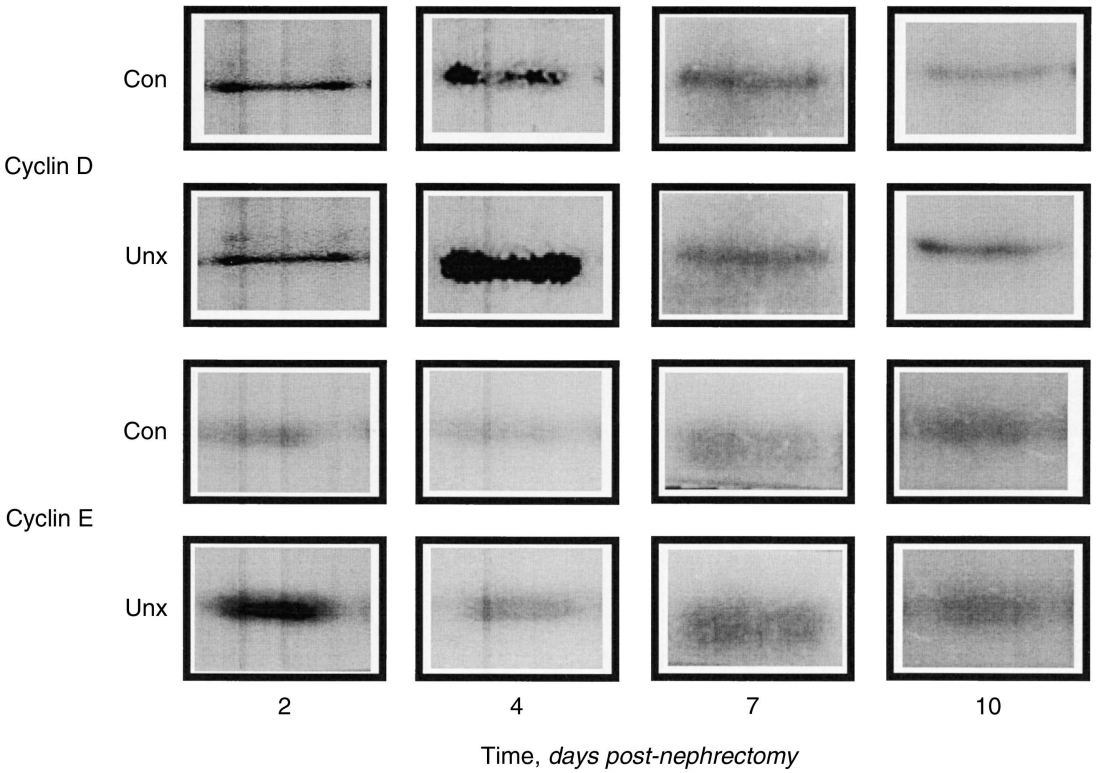

Fig. 3. G_1 kinase activity in rats post-uninephrectomy. Typical blots of cdk4/cyclin D and cdk2/cyclin E kinase activities, assayed as described in the **Methods** section.

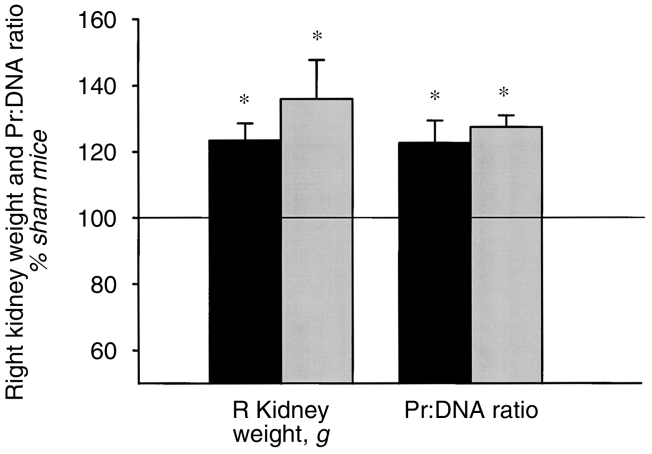

Fig. 4. Renal growth in C57Black 6 mice at 4 (■) and 7 (▩) days following uninephrectomy. Protein:DNA ratio was measured as described in the **Methods** section. $N = 6$ sham/uninephrectomy pairs for both studies at 4 days, 8 sham/uninephrectomy pairs for kidney weight at 7 days, and 4 sham/uninephrectomy pairs for protein:DNA ratio at 7 days. $^*P < 0.05$ vs. sham-operated animals.

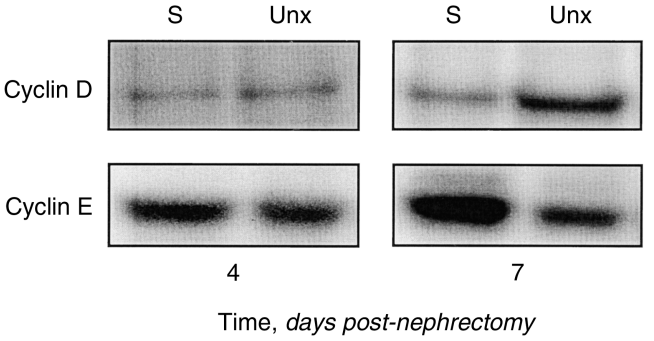

Fig. 5. G_1 kinase activity in C57Black 6 mice following uninephrectomy. Typical blots of cdk4/cyclin D and cdk2/cyclin E kinase activities, assayed as described in the **Methods** section.

enlargement is due to a hypertrophic growth process, as in the rat. However, unlike the rat, the pattern of G_1 kinase activity is more similar to that observed in diabetes mellitus and in the in vitro cell culture model of cell cycle-dependent hypertrophy [1, 5, 7]. Cdk4/cyclin D kinase activity progressively increased at 4 and 7 days [day 4, 30% increase (5/6), $N = 6$; day 7, 99% increase (8/8), $N = 8$], whereas cdk2/cyclin E kinase activity was suppressed at both time points [day 4, 38% decrease (6/6), $N = 6$; day 7: 30% decrease (7/8), $N = 8$] (Fig. 5).

Inhibition of cdk2/cyclin E kinase in rats does not prevent compensatory renal hypertrophy

In the cell cycle-dependent hypertrophy model cdk2/cyclin E kinase activity defines the growth pattern. When cdk4/cyclin D kinase activity is increased, but cdk2/cyclin

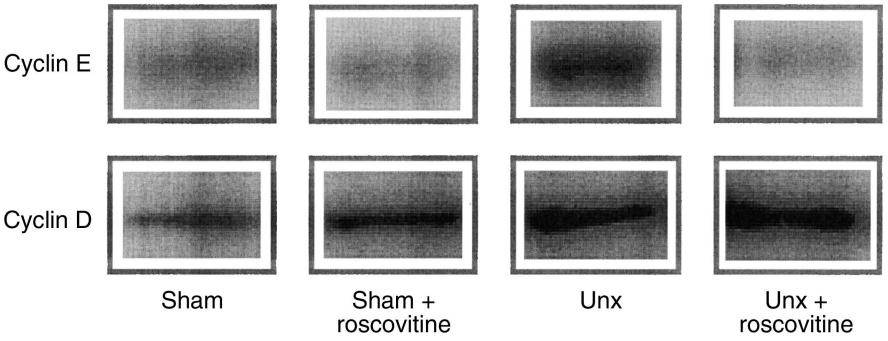

Fig. 6. Effects of roscovitine on G_1 kinase activity in rats post-uninephrectomy. Roscovitine was administered as described in the **Methods** section. Typical blots of cdk4/cyclin D and cdk2/cyclin E kinase activities, assayed as described in the **Methods** section.

Table 2. cdk2/cyclin E kinase activity

Group	BrdU incorporation[a]
Sham	1.4 ± 0.6
Sham + roscovitine	0.3 ± 0.3
Uninephrectomy	2.0 ± 0.4
Uninephrectomy + roscovitine	2.3 ± 0.8

[a] Average number of BrdU positive cells/high power field; P = NS between all groups

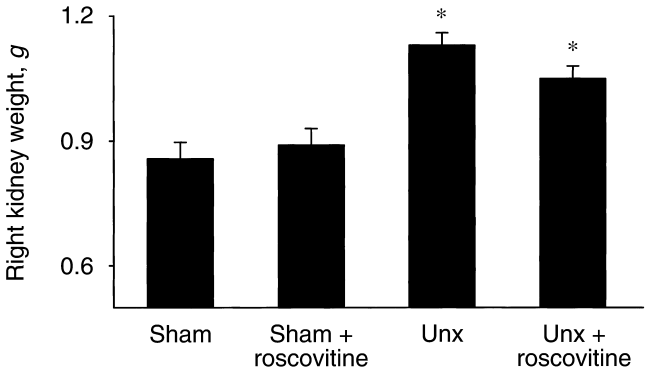

Fig. 7. Effects of roscovitine on right kidney weight post-uninephrectomy in rats. A left nephrectomy or sham surgery was performed and the right kidney harvested and weighed as described in the **Methods** section. $N = 7$ for sham, uninephrectomy, and uninephrectomy + roscovitine groups; $N = 3$ for sham + roscovitine group. $^*P < 0.05$ vs. sham-operated animals.

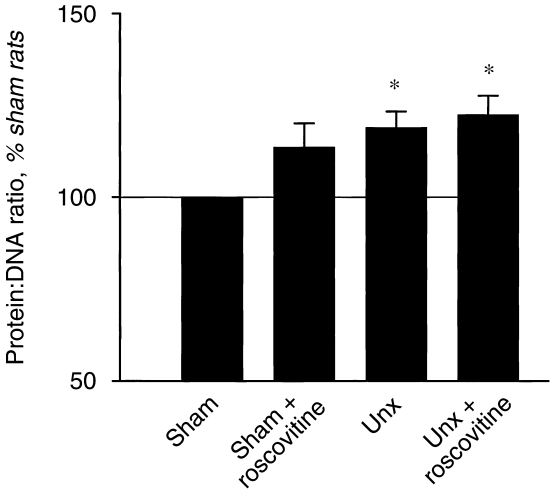

Fig. 8. Effects of roscovitine on rat isolated proximal tubule protein:DNA ratio post-uninephrectomy. Proximal tubules were isolated and the Protein:DNA ratio measured as described in the **Methods** section. $N = 9$ for sham, uninephrectomy, and uninephrectomy + roscovitine groups; $N = 5$ for sham + roscovitine group. $^*P < 0.05$ vs. sham-operated animals.

E kinase activity inhibited or activated insufficiently to reach the threshold needed to inactivate pRB and permit progression into S phase, then hypertrophy is the resulting growth pattern. In compensatory renal hypertrophy, cdk2/cyclin E kinase activity is inhibited in mice and slightly activated in rats, although without an increase in DNA synthesis (Table 1). The lack of BrdU incorporation suggests that the cdk2/cyclin E kinase activity threshold had not been achieved. To confirm that the small increase in cdk2/cyclin E kinase activity is not necessary for the hypertrophic growth response, studies were done in which cdk2/cyclin E kinase activity was inhibited with roscovitine, administered as described in the **Methods** section.

Roscovitine inhibited cdk2/cyclin E kinase activity in sham-operated and blocked the uninephrectomized-induced increase in rats [sham + roscovitine, 12% decrease (3/3), $N = 3$; uninephrectomy, 77% increase (7/7), $N = 7$; uninephrectomy + roscovitine, 9% increase (4/7), $N = 7$] (Fig. 6), and, as expected, had no effect on BrdU incorporation (Table 2). The inhibition of cdk2/cyclin E kinase activity in uninephrectomized rats had no effect on right kidney weight (Fig. 7) or the protein:DNA ratio (Fig. 8), demonstrating that the small increase in cdk2/cyclin E kinase activity in uninephrectomized rats was not necessary for the development of compensatory renal hypertrophy. This observation is consistent with the cell cycle-dependent hypertrophy mechanism, in which the role of cyclin E is to define the growth pattern. If insufficiently activated to permit progression into S phase, the growth pattern is hypertrophy.

As also shown in Figure 6, roscovitine led to a small increase in cdk4/cyclin D kinase activity in both sham and

nephrectomized rats [sham + roscovitine, 43% increase (3/3), $N = 3$; uninephrectomy, 65% increase (6/6), $N = 6$; uninephrectomy + roscovitine, 90% increase (6/6), $N = 6$] and to a small increase in the proximal tubule protein:DNA ratio in sham-operated rats. These observations suggest that cdk2/cyclin E kinase activity may play a modulating role for basal and induced cdk4/cyclin D kinase activity.

DISCUSSION

Compensatory renal growth is an adaptive response to the loss of renal mass. The present studies demonstrate that in both rats and mice compensatory proximal tubule growth following uninephrectomy is hypertrophic, not hyperplastic, and associated with regulation of the G_1 kinases in a pattern consistent with a cell cycle-dependent hypertrophy mechanism [1–6]. In this model, the development of hypertrophy requires that the cell enter G_1 and initiate events of this phase (increased protein synthesis, increased cdk4/cyclin D kinase activity, maintain pRB in its hypophosphorylated state), yet fail to progress onto S phase (absence of DNA synthesis).

The kinase activity patterns in the mice are essentially identical to the in vitro studies that characterized the mechanism, with the development of hypertrophy temporally associated with a progressive increase in cdk4/cyclin D kinase activity, and failure to activate cdk2/cyclin E kinase. This kinase profile is also the same as that observed in diabetes-induced proximal tubule hypertrophy, as is the magnitude of the kinase changes related to the hypertrophic growth [7]. In rats, the uninephrectomy-induced cell cycle kinase regulation is qualitatively the same. cdk4/cyclin D kinase activity is increased in temporal association with the development of hypertrophy. However, cdk2/cyclin E kinase activity is not inhibited, but rather slightly increased. However, the fact that (*1*) inhibition of cdk2/cyclin E kinase activity with roscovitine does not prevent the development of hypertrophy and (*2*) BrdU incorporation is not significantly increased in nephrectomized rats compared to sham-operated controls demonstrates that the slightly increased cdk2/cyclin E kinase activity is not required for the development of hypertrophy and does not induce a hyperplastic growth response. These results confirm the hypothesis that the development of hypertrophy requires that cells remain in the G_1 phase, and thus, that cdk2/cyclin E kinase activity be inhibited, remain unchanged, or not increase sufficiently to permit the cells to move into S phase.

In the rat there was a small increase in BrdU incorporation that was not statistically significant, and a small increase in cdk2/cyclin E kinase activity, neither of which were present in the mouse. This observation may represent a species difference, with a tendency in the rat to a small amount of hyperplasia post-nephrectomy.

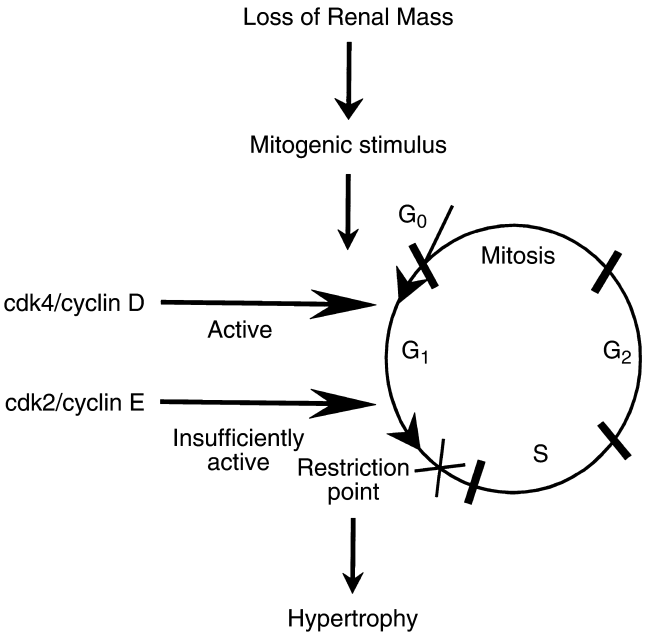

Fig. 9. A model for cell cycle-dependent compensatory renal hypertrophy.

Hypertrophic growth results in a physically enlarged cell. Cells that undergo a hyperplastic growth response also enlarge during the G_1 phase of the cell cycle. The purpose of this size increase is to ensure that at the end of mitosis each daughter cell has a full complement of the cellular material needed for survival. In hyperplasia, physical size is thought to be one of the markers indicating that the cell has successfully completed the events of G_1, and is ready to move onto the S phase [16, 17]. Hypertrophied cells can achieve a physical size larger than that achieved by cells undergoing hyperplasia. This suggests that in cell cycle-dependent hypertrophy the normal mechanisms permitting size-signaled cell cycle progression have been interrupted by the hypertrophy signal.

Thus, as schematically diagramed in Figure 9, the development of cell cycle-dependent hypertrophy requires two events to occur. First, a mitogenic signal must be activated that will move cells into the G_1 phase of the cell cycle and initiate the events of the early G_1 phase. Second, since hypertrophied cells have a single copy of DNA, cell cycle progression must be halted prior to the Restriction Point, so that the retinoblastoma protein remains active and progression into S phase is prohibited [1, 4, 6]. The cdk2/cyclin E kinase is necessary for inactivation of the retinoblastoma protein [18–20]. Thus, regulation of cdk2/cyclin E kinase activity, either by inhibition of or failure to sufficiently activate the kinase, appears to be the point at which cell cycle progression must be blocked.

In both in vitro studies and diabetes mellitus (an in vivo proximal tubule cell cycle-dependent hypertrophy model) preventing sustained cdk2/cyclin E kinase activation marks the point of interruption of the cell cycle [1, 5, 7]. The same appears to be true for uninephrectomy-induced proximal tubule hypertrophy. In models where it has been studied, failure to maintain activation of cdk2/cyclin E kinase is due to both a decrease in the number of kinase complexes that form and an increase in the abundance of cyclin kinase inhibitors (CKIs) associated with complexes that do form. Regulation of cdk2/cyclin E kinase activity by cyclin kinase inhibitors in hypertrophy has been shown in a number of renal cell types and conditions [4, 6, 21]. This has been most thoroughly studied in both in vitro and in vivo models of hyperglycemia and diabetes mellitus, respectively. In these systems, an increase in both p27 and p21, regulators of cyclin E kinase, but not p16, a regulator of cdk4/cyclin D kinase, expression is associated with mesangial cell and tubular epithelial cell hypertrophy [7, 21–27]. A role for both p27 and p21 is supported by transgenic mouse studies in which either p27 or p21 has been knocked out. In these mice, the lack of either p27 or p21 prevents mesangial cell hypertrophy [21, 28].

As mentioned, cell cycle-dependent hypertrophy requires not only a mechanism for interrupting progression through the cell cycle, but also a mitogenic signal that drives the cell into the G_1 phase, activates cyclin D kinase, and permits the growth process to begin. Preliminary studies from our laboratory suggest that the endothelin signaling system, particularly signaling through the endothelin B receptor provides that mitogenic signal in uninephrectomy-induced, but not diabetes mellitus-induced proximal tubule hypertrophy (abstract; Liu B et al, *J Am Soc Nephrol* 10:496A, 1999; and unpublished observations). Our current study shows, in contrast to wild-type mice, that in endothelin B receptor-deficient mice uninephrectomy is not associated with an increase in cyclin D kinase activity, does not result in an increase in the proximal tubule protein:DNA ratio, and thus, does not result in proximal tubule hypertrophy.

In summary, compensatory renal growth following uninephrectomy involves hypertrophy of the proximal tubule in both rats and mice. The mechanism responsible for regulating the growth process exhibits characteristics of a cell cycle-dependent mechanism (activation of cdk4/cyclin D kinase and failure to activate cdk2/cyclin E sufficiently to enter S phase), and is similar to that responsible for proximal tubule hypertrophy in diabetes mellitus.

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REFERENCES

1. Franch HA, Shay JW, Alpern RJ, Preisig PA: Involvement of pRB family in TGFβ-dependent epithelial cell hypertrophy. *J Cell Biol* 129:245–254, 1995
2. Franch HA, Preisig PA: NH_4Cl-induced hypertrophy is mediated by weak base effects and is independent of cell cycle processes. *Am J Physiol* 270:C932–C938, 1996
3. Preisig PA, Franch HA: Renal epithelial cell hyperplasia and hypertrophy. *Semin Nephrol* 15:327–340, 1995
4. Preisig PA: Renal hypertrophy and hyperplasia (chapt 27), in *The Kidney: Physiology and Pathophysiology* (vol 1, 3rd ed), edited by Selden DW, Giebisch G, Philadelphia, Lippincott Williams & Wilkins, 2000, pp 727–748
5. Liu B, Preisig PA: TGFβ-mediated hypertrophy in rat renal epithelial cells involves inhibiting pRB phosphorylation by preventing activation of cdk2/cyclin E kinase. *Am J Physiol* 277:F186–F194, 1999
6. Preisig PA: What makes cells grow larger and how do they do it: Hypertrophy revisited. *Exper Nephrol* 7:273–283, 1999
7. Huang H-C, Preisig PA: G_1 kinases and transforming growth factor-β signaling are associated with a growth pattern switch in diabetes-induced renal growth. *Kidney Int* 58:162–172, 2000
8. Jurkovitz CT, England BK, Ebb RG, Mitch WE: Influence of ammonia and pH on protein and amino acid metabolism in LLC-PK_1 cells. *Kidney Int* 42:595–601, 1992
9. Ling H, Vamvakas S, Gekle M, *et al*: Role of lysosomal cathepsin activities in cellular hypertrophy induced by NH_4Cl in cultured renal proximal tubule cells. *J Am Soc Nephrol* 7:73–80, 1996
10. Preisig PA, Alpern RJ: Increased Na/H antiporter and $Na/3HCO_3$ symporter activties in chronic hyperfiltration: A model of cell hypertrophy. *J Gen Physiol* 97:195–217, 1991
11. De Azevedo WF, Leclerc S, Meijer L, *et al*: Inhibition of cyclin-dependent kinases by purine analogues: Crystal structure of human cdk2 complexed with roscovitine. *Eur J Biochem* 243:518–526, 1997
12. Meijer L, Borgne A, Mulner O, *et al*: Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Eur J Biochem* 243:527–536, 1997
13. Pippin J, Qu Q, Meijer L, Shankland SJ: Direct in vivo inhibition of the nuclear cell cycle cascade in experimental mesangial proliferative glomerulonephritis with roscovitine, a novel cyclin-dependent kinase antagonist. *J Clin Invest* 100:2512–2520, 1997
14. Kumar AM, Spitzer A, Gupta RK: ^{23}Na NMR spectroscopy of proximal tubule suspensions. *Kidney Int* 29:747–751, 1986
15. Schaefer L, Schaefer RM, Teschner M, Heidland A: Renal proteinases and kidney hypertrophy in experimental diabetes. *Diabetologia* 37:567–571, 1994
16. Piatti S: Cell cycle regulation of S phase entry in *Saccharomyces cerevisiae. Prog Cell Cycle Res* 3:143–156, 1997
17. Zetterberg A, Larsson O, Wiman KG: What is the restriction point? *Curr Biol* 7:835–842, 1995
18. Kitagawa M, Higashi H, Jung H-K, *et al*: The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2. *EMBO J* 15:7060–7069, 1997
19. Lundberg AS, Weinberg RA: Functional inactivation of the retinoblastoma protein requires sequential modification by at least two distinct cyclin-cdk complexes. *Mol Cell Biol* 18:753–761, 1998
20. Weintraub SJ, Prater CA, Dean DC: Retinoblastoma protein switches the E2F site from a positive to negative element. *Nature* 358:259–261, 1992
21. Shankland SJ, Wolf G: Cell cycle regulatory proteins in renal disease: role in hypertrophy, proliferation, and apoptosis. *Am J Physiol* 278:F515–F529, 2000
22. Wolf G, Schroeder R, Thaiss F, *et al*: Glomerular expression of $p27^{Kip1}$ in diabetic *db/db* mouse: Role of hyperglycemia. *Kidney Intern* 53:869–879, 1998
23. Wolf G, Schroeder R, Ziyadeh FN, *et al*: High glucose stimulates

expression of p27Kip1 in cultured mouse mesangial cells: Relationship to hypertrophy. *Am J Physiol* 273:F348–F356, 1997
24. Kuan C-J, Al-Douahji M, Shankland SJ: The cyclin kinase inhibitor $p21^{WAF1, CIP1}$ is increased in experimental diabetic nephropathy: Potential role in glomerular hypertrophy. *J Am Soc Nephrol* 9:986–993, 1998
25. Wolf G, Schroeder R, Zahner G, *et al*: High glucose-induced hypertrophy of mesangial cells requires p27 (Kip1), an inhibitor of cyclin-dependent kinases. *Am J Pathol* 158:1091–1100, 2001
26. Wolf G, Stahl RAK: Angiotensin II-stimulated hypertrophy of LLC-PK_1 cells depends on the induction of the cyclin-dependent kinase inhibitor $p27^{Kip1}$. *Kidney Int* 50:2112–2119, 1996
27. Wolf G, Wenzel U, Ziyadeh FN, Stahl RAK: Angiotensin converting-enzyme inhibitor treatment reduces glomerular $p16^{INK4}$ and $p27^{Kip1}$ expression in diabetic *BBdp* rats. *Diabetologia* 42:1425–1432, 1999
28. Al'Douahji M, Brugarolis J, Brown PAJ, *et al*: The cyclin kinase inhibitor $p21^{WAF/CIP1}$ is required for glomerular hypertrophy in experimental diabetic nephropathy. *Kidney Int* 56:1691–1699, 1999