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The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction

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Chevalier RL. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction. *Am J Physiol Renal Physiol* 311: F145–F161, 2016. First published May 18, 2016; doi:10.1152/ajprenal.00164.2016.— There is an alarming global increase in the incidence of end-stage kidney disease, for which early biomarkers and effective treatment options are lacking. Largely based on the histology of the end-stage kidney and on the model of unilateral ureteral obstruction, current investigation is focused on the pathogenesis of renal interstitial fibrosis as a central mechanism in the progression of chronic kidney disease (CKD). It is now recognized that cumulative episodes of acute kidney injury (AKI) can lead to CKD, and, conversely, CKD is a risk factor for AKI. Based on recent and historic studies, this review shifts attention from the glomerulus and interstitium to the proximal tubule as the primary sensor and effector in the progression of CKD as well as AKI. Packed with mitochondria and dependent on oxidative phosphorylation, the proximal tubule is particularly vulnerable to injury (obstructive, ischemic, hypoxic, oxidative, metabolic), resulting in cell death and ultimately in the formation of atubular glomeruli. Animal models of human glomerular and tubular disorders have provided evidence for a broad repertoire of morphological and functional responses of the proximal tubule, revealing processes of degeneration and repair that may lead to new therapeutic strategies. Most promising are studies that encompass the entire life cycle from fetus to senescence, recognizing epigenetic factors. The application of techniques in molecular characterization of tubule segments and the development of human kidney organoids may provide new insights into the mammalian kidney subjected to stress or injury, leading to biomarkers of early CKD and new therapies.

acute kidney injury; atubular glomeruli; chronic kidney disease; proximal tubule; unilateral ureteral obstruction

The Challenge: a Unifying Concept of AKI and CKD

ACUTE KIDNEY INJURY (AKI) is the consequence of a series of biological processes that follow a host response to an initial renal insult (9). In the past 20 yr, the incidence of AKI has increased in parallel with an increase in end-stage kidney disease (ESKD) (99). Of greater concern, between 1976 and 1994, the prevalence of ESKD has exceeded that predicted from the prevalence of chronic kidney disease (CKD) by 70% (76). This trend has led to the recent formulation of the hypothesis that cumulative episodes of AKI culminate in CKD, and that preexisting CKD increases the risk for AKI (99). In fact, a dose-response relationship can be derived between the severity of AKI and the risk of progression of CKD (82). Moreover, the link between AKI and CKD has been established for children as well as adults (109).

Current diagnosis of AKI and CKD is limited by generally available clinical measures of kidney structure (renal ultrasound and biopsy) and function (serum creatinine concentration and urine albumin excretion). Reliance on the clearance of creatinine [or other marker of glomerular filtration rate (GFR)] as the mainstay of renal functional status is undermined by the well-established process of nephron loss accompanied by adaptive hypertrophy of remaining nephrons in CKD: a rise in serum creatinine concentration does not occur until nearly 50% of nephrons are nonfunctional (181). The observation that renal tissue from virtually all patients with ESKD is characterized by glomerulosclerosis and renal interstitial fibrosis has led to the conclusion that fibrosis plays a primary role in the process of nephron loss (37, 104, 196). As a consequence, measures of interstitial fibrosis have become the established end point for clinical studies and animal models of CKD (25, 42). As is the case for the reliance on serum creatinine concentration to detect reduced renal function, the major disadvantage of depending on renal interstitial collagen deposition to measure tissue injury is that it develops late in the response to injury. As a corollary to the unsatisfactory available biomarkers for diagnosis of kidney disease, antifibrotic therapies to prevent or to slow CKD progression have proved equally disappointing (29).

This review is an attempt to redirect the focus of renal translational research from the glomerulus and renal interstitium to the proximal tubule. First, the history leading to the current emphasis on fibrosis is examined. The central role played by unilateral ureteral obstruction (UUO) as a model of

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fibrogenesis in CKD is then considered, followed by the discovery of the generation of atubular glomeruli in this model, thereby shifting attention from the interstitium to the proximal tubule. Animal models reveal that glomerulotubular disruption and the ultimate formation of atubular glomeruli develop not only in tubular disorders, such as polycystic kidney disease (PKD) and nephropathic cystinosis, but also in "glomerular" disorders (congenital nephrotic syndrome and diabetic nephropathy). Due to its high rates of oxygen consumption and relative paucity of endogenous antioxidant defenses, the proximal tubule is particularly vulnerable to injury. Of the greatest significance, clinically relevant studies of kidney injury reveal that molecular targeting of the proximal tubule is sufficient to induce AKI, progressing to CKD when the injury is repeated. Taken together, this evidence points to the proximal tubule as the central link between AKI and CKD, and the progression of CKD is molded by the life cycle from fetus to senescence.

Focus on the Glomerulus and the Interstitium: How Did We Get Here?

Hyperfiltration: the glomerulocentric era. Recognition of the association of renal pathological characteristics with clinical signs of CKD date back to the work of Richard Bright in the early nineteenth century (16). A century later, Thomas Addis developed a clinical classification of "Bright's disease" with the collaboration of Jean Oliver, a brilliant morphologist whose elegant microdissections of nephrons from patients with CKD remain unrivaled today (2, 128). Oliver emphasized the marked heterogeneity of nephrons from patients with ESKD, with intermingling of atrophied and hypertrophied tubules, aglomerular tubules, and atubular glomeruli (Fig. 1) (128). At this time, Homer Smith (154) was making major advances in renal physiology, applying the clearance concept to elucidate glomerular and tubule function: his approach was to treat the kidney as a "black box" rather than as a collection of individual nephrons. This set the stage for the development of animal models of CKD in the second half of the twentieth century, and the formulation of the "intact nephron hypothesis" by Neal Bricker (15). Notably, Bricker concluded from glucose titration curves that there is no evidence of functional nephron heterogeneity in the diseased kidney (15). New insights were provided by the use of renal micropuncture techniques by Barry Brenner and his associates, leading to a landmark paper in 1981 (75). This study revealed that, following unilateral nephrectomy and partial ablation of the remaining kidney, remaining nephrons undergo hyperfiltration with glomerular ep-
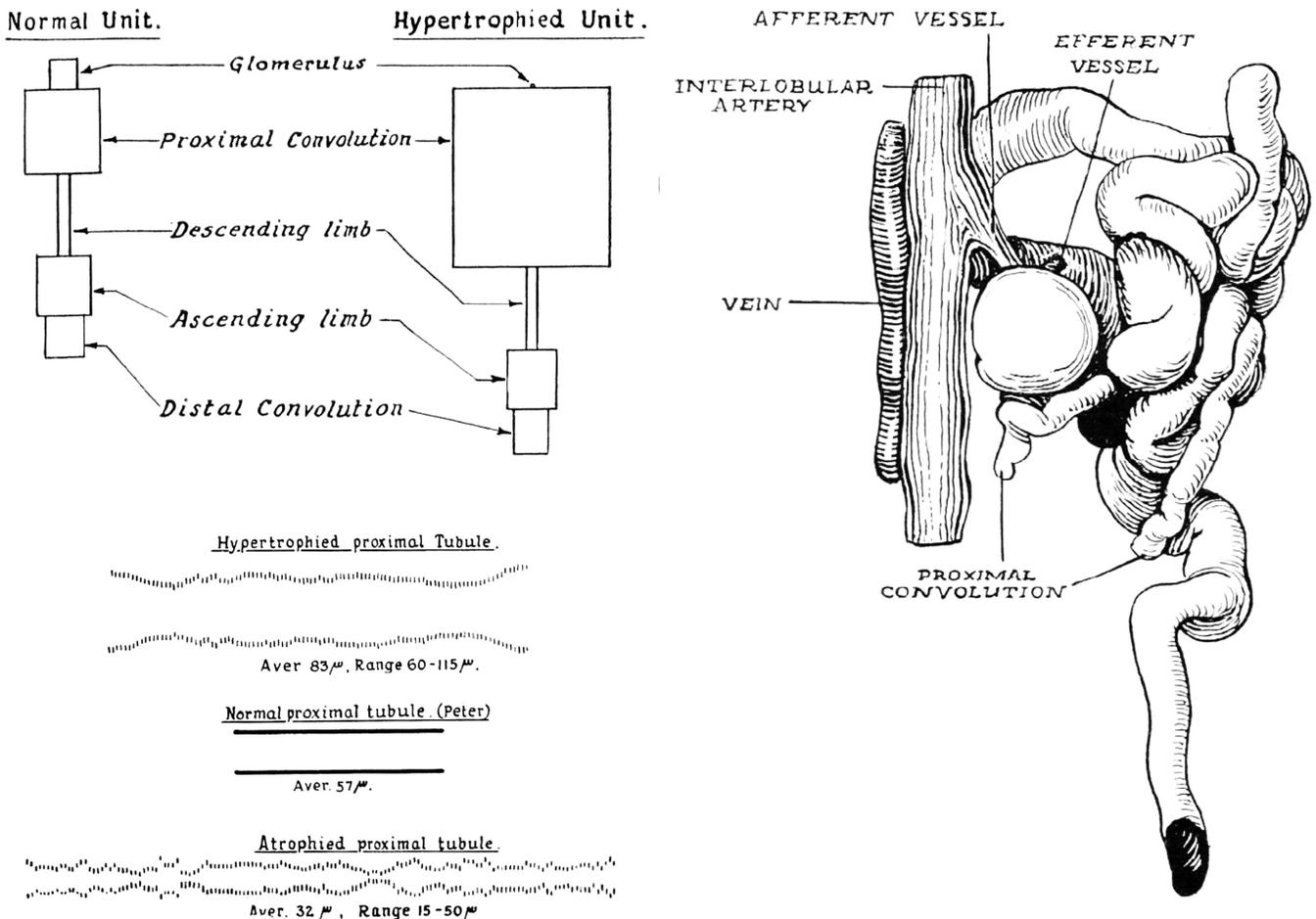
Fig. 1. Measurements of microdissected nephrons from patients with chronic Bright's disease (chronic kidney disease). *Left*, *top*: schematic normal and hypertrophied nephrons with the relative volume of glomerulus and tubular segments represented by the area of each segment. Virtually all of the adaptive growth is contributed by the proximal tubule. *Left*, *bottom*: relative proximal tubular diameter; represented by marks reflecting varying cross-sectional area along the length of the tubule. Representative hypertrophied and atrophied proximal tubular segments are compared with a normal one. *Right*: atubular glomerulus and aglomerular tubule. [Reproduced with permission from J. Oliver (128) and Wolters Kluwer.]

ithelial and mesangial injury: these changes are markedly attenuated by a low-protein diet (75). The authors concluded that sustained glomerular hyperfiltration is a maladaptive response, preserving whole-kidney GFR at the expense of progressive nephron injury. This work launched decades of studies of mechanisms underlying glomerular hyperfiltration and hypertrophy, as well as resulting glomerulosclerosis: the glomerulocentric era was born.

The concurrent development of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers resulted in their use as therapeutic agents to prevent or slow the hyperfiltration response in animal models of CKD (142). The United States Food and Drug Administration approved the use of this therapy in diabetic nephropathy, and it has found widespread use in attempting to slow virtually all forms of CKD, although with limited effectiveness (19). The hyperfiltration theory predicted that reduction in nephron number, regardless of etiology, is a major risk factor for progression of CKD (13).

Unilateral ureteral obstruction: focus on the interstitium. Over the past 3 decades, complete UUO has become the most widely used animal model of progressive CKD, with the annual number of publications increasing from 10 in 1986 to 100 in 2015 (Fig. 2) (38). Surgical ligation of a ureter is a simple procedure: it rapidly results in nephron injury with characteristics of human ESKD, and its application to murine genetically engineered mutants can be used to target the roles of specific genes and cell types in the kidney (8, 25). This work has been fueled by the rapid development of molecular techniques and reagents that led to the identification of many cellular pathways and interactions that play a role in injury to the obstructed kidney (22). As noted above, because renal interstitial fibrosis is the hallmark of ESKD, the end point of most of the studies targeted renal interstitial cellular infiltrates [monocytes/macrophages and α-smooth muscle actin (a marker of myofibroblasts)] and interstitial collagen deposition (25). Our laboratory has used rodent models of UUO for over 30 yr, with a primary focus on the neonatal period, seeking insight into the pathogenesis of congenital urinary tract obstruction, the primary identifiable cause of CKD in the pediatric population (119). These studies revealed tubule cell death as well as altered renal expression of growth factors and cytokines. These include components of the renin-angiotensin system, transforming growth factor-β (TGF-β), and adhesion
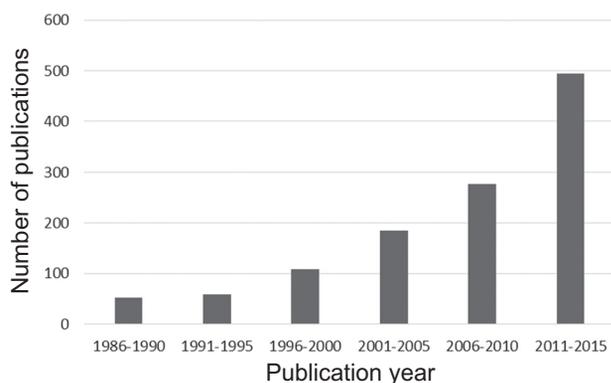

Fig. 2. Number of papers containing the key words "unilateral ureteral obstruction" published between 1986 and 2015. Medline search was performed April 2016. There was a 10-fold increase in the annual number of publications between 1986 and 2015.

molecules (26–28, 40, 44, 95, 96, 126, 189, 190, 193). Although the model of unilateral ureteral ligation provides a convenient assay for quantitation of interstitial fibrosis, complete UUO rarely occurs in the clinical setting and represents an extreme insult to the kidney. To better reproduce congenital obstructive nephropathy, we have developed models of reversible variable partial UUO, which allows independent manipulation of the timing, severity, and duration of obstruction (150, 167, 168, 191, 192).

Shifting the Focus to the Proximal Tubule

Serendipity: the glomerulotubular junction and atubular glomeruli. In the course of a study of the renal cellular recovery following release of partial UUO in the neonatal mouse (168), we were preparing to investigate the role of endothelial nitric oxide synthase (eNOS) in obstructive injury, using eNOS knockout mice (51). However, kidneys of sham-operated adult eNOS mutant mice exhibited macroscopic indented scars containing crowded small glomeruli that proved by serial sectioning to be disconnected from their proximal tubules (51). Examination of neonatal eNOS knockout mouse kidneys revealed apoptosis and necrosis of focal wedge-shaped areas of renal parenchyma, containing nephrons with narrowing of the glomerulotubular junction (51). This led to the conclusion that endogenous renal eNOS production contributes to the maintenance of nephron maturation and integrity. Of note, human eNOS gene polymorphisms are associated with reduced enzyme activity and the development of ESKD (124). Upon reexamination of kidneys in the neonatal partial UUO study, areas of proximal tubule cell death and glomerulotubular disconnection were discovered; release of UUO arrested this process, with remodeling of the renal architecture (Table 1) (168).

These observations prompted a review of the literature, which revealed that injury to the glomerulotubular junction and formation of atubular glomeruli has been described in a wide variety of renal disorders, including glomerular diseases (IgA nephropathy and diabetic nephropathy), tubular diseases (renal allograft rejection, obstructive nephropathy, pyelonephritis, and PKD), and toxic nephropathies (adriamycin, cisplatin, and lithium) (24). Using microdissection in the 1930s, Jean Oliver (128) had described atubular glomeruli (and aglomerular tubules) in kidneys from patients with Bright's disease (Fig. 1). Subsequently, in the 1990s, using serial sectioning, Niels Marcussen (111) described atubular glomeruli in patients with CKD due to a variety of disorders. It is likely that glomerulotubular injury and the formation of atubular glomeruli has remained largely underappreciated due to the labor-intensive nature of microdissection and serial sectioning of tissue. A recent study demonstrated the use of multiphoton microscopy to visualize atubular glomeruli in situ in a murine model of cisplatin nephropathy, an approach that could significantly reduce the time and effort required to quantify the fraction of atubular glomeruli (170).

Unilateral ureteral obstruction revisited. In light of this information, we implemented histomorphometry to investigate the most commonly used animal model of CKD: 7 and 14 days of complete UUO in the adult mouse (48). This revealed that, compared with sham-operated animals, relative vascular volume fraction remained unchanged, and interstitial matrix com-

Table 1. *Segmental proximal tubular function and response to injury: animal models and human diseases*

Model/Disease	Glomerulotubular Junction Response To Injury	Proximal Tubule	Proximal Tubule Energy
Renal ablation	ATG early (58)	Progressive proximal tubular atrophy → ↑ ATG from 9 to 48%, 10–25 wk after 5/6 nephrectomy	↑ Q_{O_2}, early (121)
Unilateral ureteral obstruction	ATG early (50)	Oxidative stress and cell death by autophagy, apoptosis, necrosis	↓ Q_{O_2}, ↑ glycolysis (158)
Polycystic kidney disease	ATG late (56)	Oxidative stress and cell death	
Congenital nephrotic syndrome	ATG early (176)	Protein overload proximal tubule injury	
Cystinosis	ATG late (55, 97)	Swan neck. Mitoquinone protective (antioxidant) (55)	↓ mitochondria (55)
Diabetes	Type 1 → ATG early (117)	71% GTJ abnormalities in proteinuric type 1 diabetics	↑ catalase protective in mouse (14), mitochondrial uncoupling and
	Type 2 → ATG late (186)	44% GTJ abnormalities in type 2 diabetic patients with low proteinuria	↑ Q_{O_2} →hypoxia (52)
Renal artery stenosis	Mild-reversible (73)	Tubular atrophy and thickened TBM reverse with unclipping and stopping ACE inhibitor (73)	↓ mitochondria ↓ Na-K-ATPase
	severe → ATG late (110)	52% ATG, 40% glomeruli connected to atrophic tubules; normal number of glomeruli (110)	
Chronic allograft rejection	ATG late	18% ATG 7 yr posttransplant (133)	Rat renal transplant → mitochondrial uncoupling, ↑ Q_{O_2} → hypoxia (134)

Nos. in parentheses, reference nos. ATG, atubular glomeruli; GTJ, glomerulotubular junction; TBM, tubular basement membrane; ACE, angiotensin-converting enzyme; Q_{O_2}, oxygen consumption.

ponents did not exceed 15% of the total volume fraction of the obstructed kidney (50). In contrast, there was a very rapid loss of renal parenchyma in the completely obstructed kidney, due to a 65% reduction in proximal tubule mass (50). Proximal tubules underwent oxidative injury, apoptosis, necrosis, and autophagy, with widespread mitochondrial loss and tubular collapse (50). After 14 days of UUO, this was associated with 46% of glomeruli developing atrophic proximal tubules at the glomerulotubular junction, and 39% becoming atubular (Table 1) (48). Thus, based on sequential quantitative morphology, rather than interstitial expansion or fibrosis, the primary casualty of obstructive injury is massive proximal tubule cell death initially localized to the glomerulotubular junction. In contrast to the formation of collapsed proximal tubule fragments, epithelial cells at the urinary pole of Bowman's capsule undergo transition to a mesenchymal phenotype and extend to seal the capsule while maintaining perfusion of the glomerular tuft, with recruitment of renin-expressing cells down the afferent arteriole (48). Progenitor cells have been identified at the urinary pole of Bowman's capsule, and these may play a role in its repair (139), allowing the glomerulus to continue its endocrine function by production of renin, a homeostatic response (149). These events suggest that, rather than primary interstitial signaling inducing fibrotic pathways, both regenerative and degenerative processes are activated in the proximal tubule by UUO, and that these are drivers of additional nephron loss. This conclusion is supported by a recent study in which two proapoptotic members of the Bcl-2 family were investigated in mice subjected to 7 days of complete UUO (84). Mice with either proximal tubule-specific Bax deletion or systemic deletion of Bak had no effect on obstructive renal injury, whereas combined deletion of Bax and Bak inhibited tubule apoptosis and atrophy, as well as secondary interstitial inflammation and fibrosis: targeting the fate of proximal tubule cells determines the downstream interstitial consequences of

UUO (84). Underscoring the importance of long-term follow up in animal models, an earlier study of rats 10 and 25 wk following renal ablation revealed tubule atrophy and formation of atubular glomeruli that preceded the development of glomerulosclerosis; at 25 wk, 48% of glomeruli were atubular, whereas only 14% were globally sclerotic (Table 1) (58).

Allometry of the proximal tubule: limits of growth and senescence. Allometry, or the study of differential growth of components of an organism's morphology, tends to be forgotten in the pursuit of cellular and molecular mechanisms. However, the functional consequences of changes in nephron size are of central importance: metabolic rate is the primary determinant of GFR (151). This becomes particularly relevant in relating the myriad metabolic activities of the proximal tubule to segments of the nephron, as well as individual cells (185). For example, in addition to proliferation and hypertrophy, the proximal tubule cells must elaborate a brush border to maximize reabsorptive surface area, a feature that is evolutionarily conserved from the pronephros of fish to the metanephros of mammals (140, 185). At present, the gene regulatory networks responsible for orchestrating proximal tubular size remain largely unknown.

The proximal tubular mass constitutes over 50% of the volume of the normal kidney, and growth of the proximal tubule accounts for the increasing size of the kidney from late gestation through early childhood (23). Proximal tubular length increases from 2 mm at birth to 12 mm in the adult, and its rate of growth outstrips that of the glomerulus (Figs. 3 and 4) (45). Because nephron maturation proceeds centrifugally, the longest proximal tubules lie in the inner cortex, whereas the shortest are in the subcapsular region: nephron heterogeneity is extreme in the neonate, with the ratio of longest to shortest being greater than 10:1 (45). By 1 mo of age, internephron variation decreases with a ratio of only 3.5:1, and by adulthood decreases further to 1.5:1 (45). The reduction in proximal
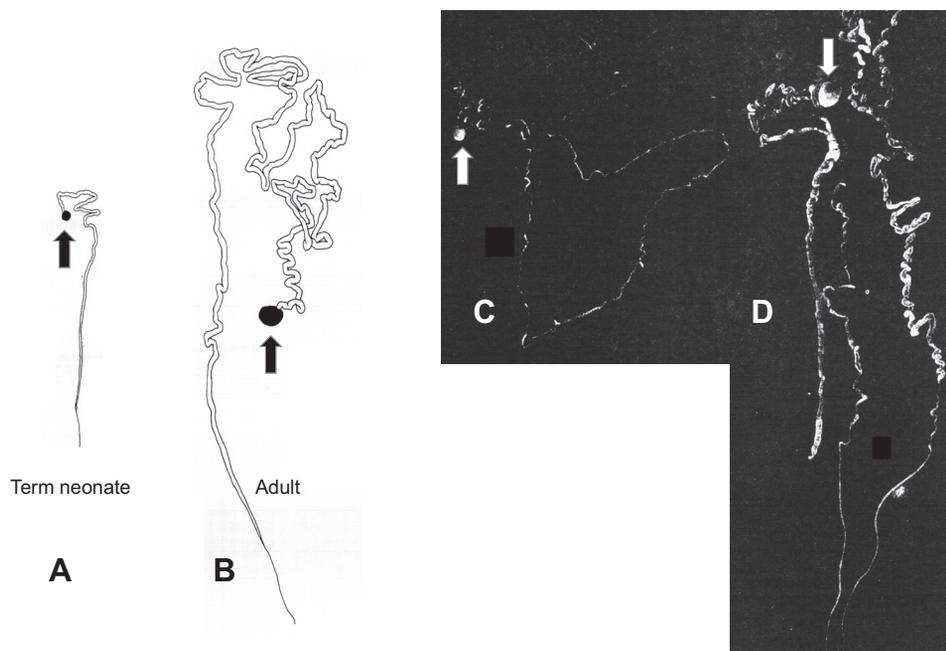
Fig. 3. Microdissected human nephrons. *A* and *B*: glomerulus and proximal tubule from term neonate and adult, respectively. *C*: complete atrophic nephron from aging adult. *D*: complete hypertrophic nephron from aging adult. Arrows indicate glomeruli. [Reproduced with permission from J. Oliver (129; 132) and Wolters Kluwer.]

tubule mass with aging is due not only to nephron loss (127), but also to increasing nephron heterogeneity, with the formation of two populations of nephrons, those that are atrophied and those with adaptive hypertrophy, a pattern also characteristic of CKD (Figs. 1 and 3) (128, 132). Proximal tubule hypertrophy can be a homeostatic response to reduced nephron number due to genetic disorders (144), to nephron loss (as in CKD) (130), or to chronic hyperglycemia (as in diabetes, see below) (175).

Maximal tubular size is limited by the dimensions (luminal diameter and tubular length) that create low enough resistance to allow tubular fluid flow for adequate reabsorption. This is solved in kidneys of the whale by nephrons being packed in many small clusters (renicules) (108). These considerations become important in understanding the response to injury of the newborn kidney, or in devising strategies to enhance regeneration in the mature kidney: physical constraints dictate the minimum number of nephrons that can maintain homeostasis. As noted above, congenital obstructive nephropathy is the primary cause of CKD in children and is often accompanied by reduced nephron number, an independent risk factor for adult CKD (105). To determine the role of nephron number on the long-term recovery following the release of obstruction in neonatal mice, we compared wild-type mice to *Os/+* mice that are born with a 50% reduction in number of nephrons (150). Whereas release of obstruction allowed proximal tubular
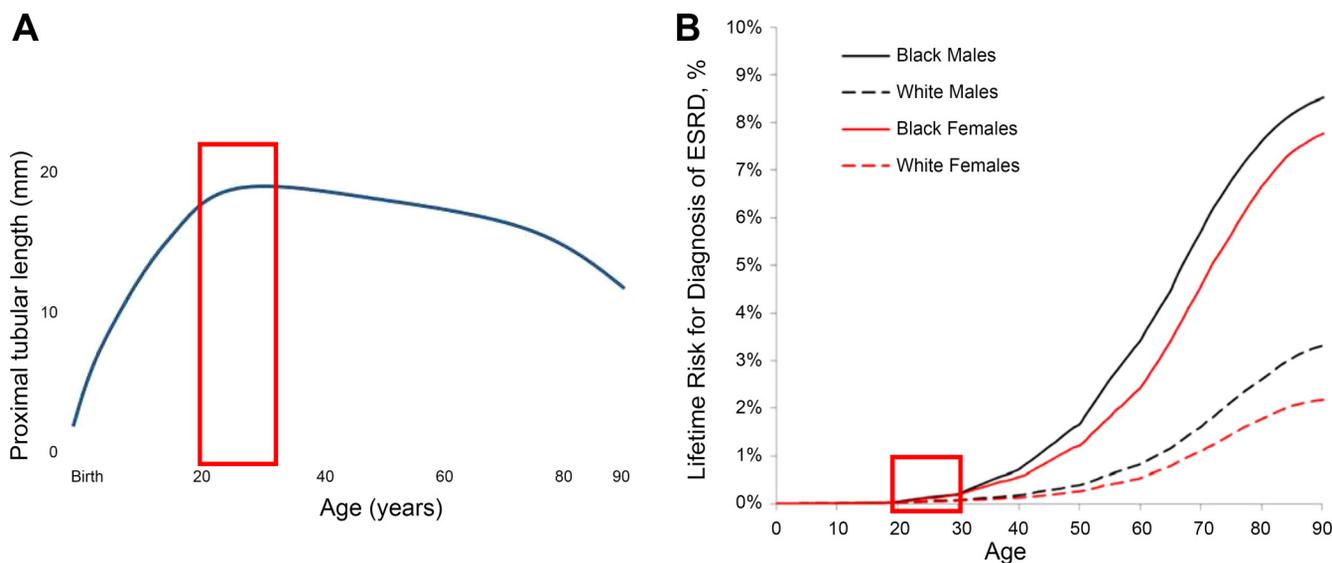
Fig. 4. *A*: length of human proximal tubule from birth to 90 yr. Data are shown from measurements of microdissected nephrons from 105 cadavers undergoing routine necropsy, who had suffered acute death. Steady growth from birth to 20 yr is followed by a plateau and a gradual decline after the 3rd to 4th decade. Red box = 20–30 yr of age, peak reproductive years. *B*: lifetime risk for diagnosis of ESKD from birth to 90 yr, from Grams et al. (68). [Graphic representation of data in *A* reproduced with permission from Darmady et al. (33) and Wiley.]

growth to resume in wild-type mice, growth was not restored in *Os/+* mice, which also underwent additional nephron loss (150). These findings suggest that infants with low nephron number (such as very-low-birth-weight infants) are at increased risk for progressive CKD due to obstructive nephropathy.

A provocative study of renal allograft mass-to-recipient size ratio in pediatric transplanted kidneys revealed that 24 mo posttransplantation, transplants of lower graft mass demonstrated increasing renal volume, whereas those of higher graft mass actually decreased by a similar amount (43). The resistive index decreased throughout early follow-up in patients receiving transplants of lower graft mass, whereas an increase was noted in those receiving organs of high graft mass, suggesting inadequate perfusion of the larger grafts (43). These observations suggest that, in addition to immunological incompatibilities, a mismatch of physical organ size can influence graft outcome. Despite decades of investigation, the determinants of compensatory renal growth remain to be elucidated.

Following very rapid growth in proximal tubular length in the first 2 decades of life (45), tubule length gradually begins to shrink after 40 yr of age (Fig. 4*A*) (33). In a recent study, kidney volume was measured by computed tomography in 1,344 potential kidney donors 18–75 yr of age (182). Consistent with the earlier microdissection study (33), reduction in renal parenchymal volume with age was correlated with decreasing GFR and decreasing cortical volume (a reflection of proximal tubular mass), but slightly increasing medullary volume (182). Notably, albuminuria was associated with increasing parenchymal volume in apparently healthy adults, suggesting hypertrophy of some proximal tubules in hyperfiltering nephrons.

*Responses of the Proximal Tubule to Injury:
a Varied Repertoire*

The cumulative incidence of ESKD remains below 1% until after 40 yr of age, with significantly greater subsequent increases in men than women and in black than white patients (Fig. 4*B*) (68). Race and sex disparities in CKD remain important epidemiological concerns and have been recently reviewed (125). Unlike men or postmenopausal women, in naturally ovulating women, urinary excretion of proximal tubule-specific enzymes peaks within 7 days after ovulation or onset of menses (148). These findings indicate novel proximal tubular adaptive responses to the normal human female reproductive hormone cycle, consistent with recurring increases of tubular cell turnover that may confer resistance to tubular injury (148). Notable is the increase in CKD after the peak reproductive period (20–30 yr) (Fig. 4*B*), which coincides with the decrease in proximal tubular length (Fig. 4*A*). There is a shift in the primary diagnoses for CKD in adults compared with children: congenital anomalies of the kidneys and urinary tract contribute >50% of cases in children (with obstructive nephropathy the leading cause) (119), whereas diabetes and hypertension predominate in adults (174). It is now apparent, however, that, for most children with congenital anomalies progressing to ESKD, renal replacement therapy is delayed until adulthood (188). There is marked heterogeneity in the rate of progression of kidney disease over the life span, and the proximal tubule displays a variety of responses to injury,

ultimately leading to tubule atrophy, glomerulotubular disruption, and formation of atubular glomeruli in glomerular, tubular, and toxic nephropathies (see Table 1 in Ref. 24). Renal morphometric analysis by serial sectioning reveals that, in nephropathic cystinosis, a rare inherited disorder of cystine transport that leads to ESKD in the second decade of life, there is a preponderance of atubular glomeruli by 10–24 yr of age (Fig. 5) (97). In contrast, diabetic nephropathy progresses less rapidly, develops later in life, and results in a greater proportion of atubular glomeruli in type I than type II, which spans later adulthood (Fig. 5) (117, 186).

Recent experimental studies have elucidated a remarkable variety of mechanisms leading to proximal tubular injury and progression of CKD. To illustrate these, four disorders are examined below: PKD and nephropathic cystinosis ("tubular" disorders), and congenital nephrotic syndrome and diabetic nephropathy ("glomerular" disorders).

Polycystic kidney disease: the proximal tubule as collateral damage. The leading monogenic cause of CKD, autosomal dominant PKD is characterized by the slowly progressive increase in the size of scattered cysts that arise from proximal and distal tubules (171). Expanding cysts encroach on neighboring intact nephrons, and, with continued exponential enlargement, remaining proximal tubular volume is progressively reduced, leading to ESKD (20). The *pcy* mutant mouse develops progressive PKD with markedly increasing cyst size by 30 wk of age. Morphometric study of *pcy* compared with wild-type mice revealed a linear relationship between fractional cyst area and damage to the glomerulotubular junction (56), a measure of severity of urinary tract obstruction (see above). This is paralleled by a progressive increase in the fraction of glomeruli attached to atrophic tubules, as well as atubular glomeruli (56). Tubule apoptosis is present in patients with PKD and *pcy* mice, as in mice subjected to UUO (48, 187). Examination of kidneys from patients with early- and late-stage PKD revealed a progressive increase in the fraction of glomeruli attached to atrophic tubules and atubular glomeruli (Table 1) (56). These findings demonstrate important parallels between the UUO model and PKD and support efforts to
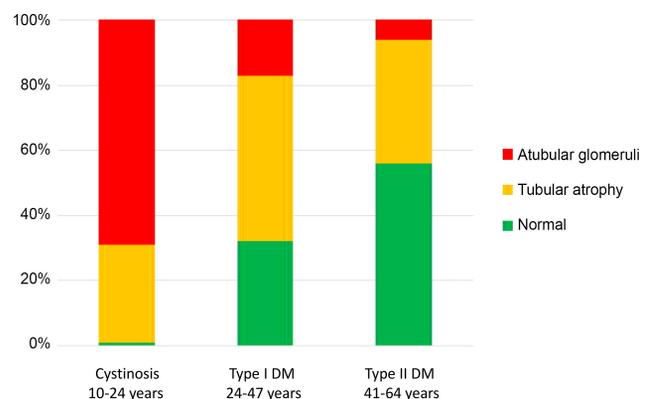
Fig. 5. Fractional distribution of glomerulotubular junction integrity in serial sections of glomeruli from patients with nephropathic cystinosis and diabetic nephropathy. Data are arranged by age group, with age range for patients in each diagnostic group. "Atubular glomeruli" are those with no tubular connection; "Tubular atrophy" indicates glomeruli connected to atrophic tubules; and "Normal" indicates glomeruli connected to normal tubules. DM, diabetes mellitus. Data were derived from Larsen et al. (97), Najafian et al. (117), and White et al. (186).

develop therapies to slow the growth in cysts in patients with PKD. Superior to available molecular markers, total cyst volume is currently the best predictor of progression of the disease and may serve as a surrogate for nephron loss due to tubular obstruction (20).

Nephropathic cystinosis: the "swan-neck lesion" as an adaptive strategy. Nephropathic cystinosis is a rare congenital metabolic disorder resulting from a mutation in cystinosin, a lysosomal cystine carrier, which leads to severe Fanconi syndrome and progressive CKD (122). An attenuated glomerulotubular junction ("swan-neck lesion"), the characteristic renal histological feature, develops between 6 and 12 mo of age (Fig. 6) (107). By the onset of renal failure between 10 and 25 yr of age, most glomeruli are atubular, the result of chronic proximal tubular injury due to cystine accumulation (Fig. 5) (97). An animal model of nephropathic cystinosis, the *CTNS* knockout mouse, develops glucosuria, phosphaturia, and progressive CKD, all of which mirror features of human cystinosis (123). Detailed studies of proximal tubular changes during the life cycle of $CTNS^{-/-}$ mice revealed that, rather than representing atrophy, epithelial cells at the leading edge of the swan-neck lesion (beginning at the glomerulotubular junction) undergo progressive loss of mitochondria and expression of megalin Transfer as shown, consistent with dedifferentiation, and undergo extreme flattening with thickening of the underlying tubule basement membrane (54, 55). Whereas flattened cells of the swan-neck lesion resist uptake of filtered cystine (due to loss of megalin and mitochondria), the narrower tubular diameter and thickened basement membrane resist distension that would result from high hydrostatic pressure present in the S1 segment (the glomerular tuft remains normal) (55, 143, 184). Notably, epithelial cells of the downstream S3 segment compensate for the thinning S1 segment by increasing endocytosis

with subsequent lysosomal clearance of cystine into tubular fluid (54). Initiation of the swan-neck lesion is at least in part dependent on proximal tubule oxidative injury resulting from cystine accumulation: administration of mitoquinone, an antioxidant targeted to mitochondria, significantly delays extension of the lesion (55). The adaptive responses of the proximal tubule ultimately fail, with clusters of proximal tubules exhibiting oxidative stress, upregulation of kidney injury molecule-1 (KIM-1), and activation of inflammasomes (55, 136). As is the case in human cystinosis, the terminal event in $CTNS^{-/-}$ mice is the formation of atubular glomeruli and interstitial fibrosis (55) (Table 1, Figs. 5 and 6).

Congenital nephrotic syndrome: overwhelming proximal tubule albumin reabsorption. Congenital nephrotic syndrome usually results from mutations coding for proteins essential to the podocyte slit diaphragm, resulting in massive proteinuria and hypoalbuminemia (138). While generally regarded as a purely glomerular disorder, microdissection of nephrons from a 2-mo-old infant with congenital nephrotic syndrome revealed narrowed glomerulotubular junctions, with cystic dilatation of the downstream portion of the proximal tubule (46). Examination of serial histological sections of kidneys from seven additional patients with congenital nephrotic syndrome revealed 30% atubular glomeruli, which were ascribed to heavy proteinuria (Table 1) (176). Kriz and LeHir (93) propose two mechanisms for the proximal tubular response to proteinuria: *1*) direct encroachment of extracapillary lesions, resulting from primary glomerular injury, formation of crescents, and misdirected glomerular filtrate and obstruction of the glomerulotubular junction; and *2*) leakage of proteins through injured glomeruli, leading to excessive protein reabsorption by proximal tubules and secondary tubular degeneration. Contrary to long-held views, it appears that the proximal tubule can re-
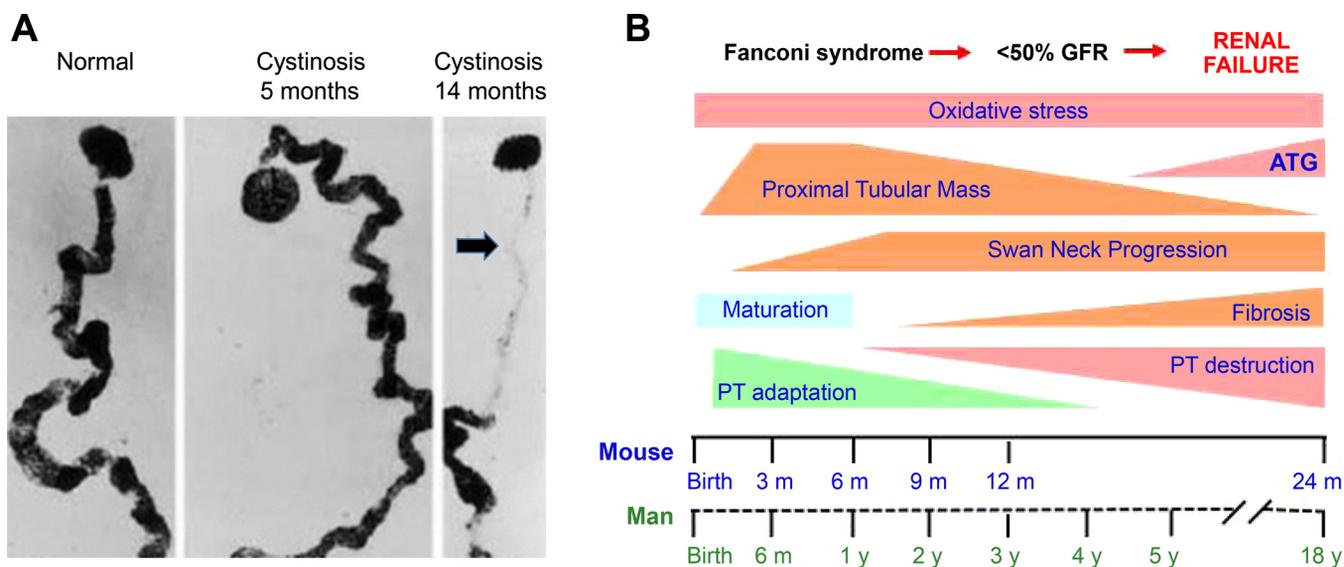

Fig. 6. Progression of cystinosis in mouse and man. *A*: "swan neck deformity" of the proximal tubule in human cystinosis develops after 5 mo of age is shown: normal, cystinosis 5 mo, and cystinosis 14 mo. *B*: scheme showing nephron changes with age in murine and human cystinosis. Fanconi syndrome generally develops in the first 6 mo of life, with significant decrease in GFR by 2–5 yr, and renal failure in the 2nd decade. There is marked interindividual variability in the rate of progression of renal lesions, and treatment with cysteamine can significantly delay progression to renal failure. The evolution of renal lesions in the mouse is more gradual, with GFR decreasing only later in adulthood. The period of proximal tubular maturation extends through the first 6 mo in humans and the first 3 mo in the $Ctns^{-/-}$ mouse; the "swan-neck lesion" is initiated in the first 3 mo in the mouse, and between 6 and 12 mo in humans. The development of irreversible renal lesions in both species includes loss of functional proximal tubular mass and the development of tubular atrophy, interstitial fibrosis, and formation of atubular glomeruli. Therapy should be directed to the early period of adaptation (potentially reversible changes) rather than the late (destructive) phase. [Reproduced with permission from Mahoney and Striker (107) and Springer, and Galarreta et al. (55).]

claim large amounts of filtered albumin by transcytosis mediated by the neonatal Fc receptor pathway in concert with megalin, a receptor in the tubulovesicular system below the apical membrane of proximal tubule cells that serves as an endocytic scavenger of filtered proteins (36). Comparison of two models of protein overload reveals a surprising capacity of the proximal tubule to regulate uptake of albumin (180). Acute exogenous albumin overload is followed by a significant decrease in proximal tubular albumin uptake, whereas targeted deletion of podocytes leads to hypoalbuminemia and markedly increased proximal tubule uptake (180). The latter response presumably contributes to progressive proximal tubule injury in patients with congenital nephrotic syndrome. The role of megalin in this context has been revealed in double transgenic mice lacking proximal tubule megalin and podocyte depletion that develop massive proteinuria but mild glomerular and tubule injury (114). Comparison of megalin-containing to megalin-deficient proximal tubule cells showed that albumin and immunoglobulins were preferentially accumulated in megalin-containing cells, which also preferentially expressed heme oxygenase-1 (HO-1) and monocyte chemotactic protein-1, and underwent apoptosis (114). A prior study using a similar experimental approach, but in which glomerular injury was induced by anti-glomerular basement membrane antibody, concluded that tubular lesions arise from direct extension of glomerular lesions down the glomerulotubular junction (166). These two studies garner support for both mechanisms proposed by Kriz and LeHir (93), which are not mutually exclusive (30). Although inhibition of megalin-mediated proximal tubular transcytosis would appear to be an effective therapy to reduce proximal tubular injury due to glomerular protein leakage, megalin is necessary for proximal tubular uptake of survivin, an antiapoptotic molecule that regulates cell division and survival (85). There is clearly much to be learned regarding the role of the proximal tubule in maintaining albumin homeostasis: this function must be balanced with multiple other vital homeostatic functions, such as reclamation of sodium, bicarbonate, phosphorus, amino acids, and glucose (118).

Diabetic nephropathy: a disorder of the proximal tubule. Diabetes is the major cause of CKD in adults, and diabetic nephropathy has been viewed primarily as a glomerular disorder, resulting in glomerulosclerosis. There is evidence, however, for the earliest responses of the nephron being centered in the proximal tubule rather than the glomerulus. Proximal tubule cells cannot decrease glucose transport rates enough to prevent increased intracellular glucose in the face of hyperglycemia (175). This results in hypertrophy of proximal tubule cells, hyper-reabsorption, and hyperfiltration due to tubuloglomerular feedback, and increased production of reactive oxygen species leads to oxidative stress and increased production of angiotensinogen (6, 175). Initially, proximal tubule cells undergo hyperplasia with increased production of TGF-β, leading to G1 cell cycle arrest, hypertrophy, and a senescence phenotype (175). Transformed proximal tubule cells, in turn, stimulate interstitial inflammation with myofibroblast-initiated collagen deposition (175). Of note, morphometric study of kidneys from patients with type 1 or type 2 diabetes reveal widespread glomerulotubular junction abnormalities and formation of atubular glomeruli (Table 1, Fig. 5) (116, 186). Nondiabetic transgenic mice overexpressing angiotensinogen

in proximal tubules develop albuminuria, tubular apoptosis, and interstitial fibrosis (64). These sequelae are prevented in double-transgenic mice that overexpress both catalase and angiotensinogen in proximal tubule cells (64). Streptozotocin-induced diabetic mice transgenically overexpressing catalase in proximal tubule cells reveal that proximal tubular oxidative stress plays a central role in the generation of angiotensinogen and apoptosis, hallmarks of diabetic nephropathy (6, 14).

Proximal Tubule Function, Injury, and Energy Conservation

Overlapping mechanisms in AKI and CKD. Abnormalities of renal perfusion have been shown to play a significant role in both AKI and CKD. The interplay between impaired production of vascular endothelial growth factor by damaged proximal tubules and peritubular rarefaction likely plays an important role in AKI-CKD transition (163). Four weeks following microembolisms induced by intra-arterial injection of microspheres in intact adult rats, foci of atrophic tubules appear, some of which contain a mosaic distribution of both atrophic and normal-appearing epithelial cells (159). The tubules are surrounded by damaged peritubular capillaries and interstitial cells expressing platelet-derived growth factor receptor-β and α-smooth muscle actin (159). These data indicate that ischemia-induced hypoxia can impair the renal microcirculation, leading to focal proximal tubular injury that, in turn, results in interstitial inflammation and fibrosis (18). In progression of CKD, injured tubule cells with impaired production of vascular endothelial growth factor are more likely to be the cause rather than the consequence of capillary rarefaction (177).

As is the case for CKD, the pathophysiology of AKI is complex and involves all components of the nephron. Ischemia-reperfusion is a common cause of AKI, and micropuncture studies have shown that tubular obstruction is a major factor in its pathogenesis, whereas tubular repair with relief of obstruction are early events in the recovery from injury (5, 47). However, in contrast to the UUO model, in which the glomerulotubular junction appears to be a major target of injury and repair, the proximal tubule S3 segment is most susceptible in AKI (Table 2). Targeted ablation of this segment in transgenic mice results in polyuria followed by oliguria, with exfoliation of S3 segment cells into the tubular lumen (147). It appears that S1 proximal segments act as sensors of injurious stimuli and modulate the response of S2 and S3 segments to injury by signaling (cross talk) between segments (39). Unlike S2 and S3 segments, the S1 segment lacks peroxisomes, organelles that detoxify accumulated fatty acids but can be a source of reactive oxygen species in injured cells (39). By contrast, like macrophages, S1 segment cells protect themselves from oxidative injury by upregulation of HO-1 and sirtuin 1 (Table 2) (39, 87). Administration of an inhibitor of heme oxygenase aggravates kidney dysfunction in a model of glycerol-induced AKI, revealing the importance of endogenous HO-1 in proximal tubule protection (120). A subfamily of nuclear receptor transcription factors, peroxisome proliferator-activated receptors (PPARs), appear to play a significant role in the proximal tubular response to injury. Chronic UUO reduces kidney PPAR-α expression in mice, and transgenic expression of proximal tubule PPAR-α reduces production of TGF-β and proinflammatory cytokines following UUO, and also reduces oxidative injury

Table 2. *Segmental proximal tubular function and response to injury: mechanisms*

Function	Glomerulo-Tubular Junction	S1	S2, S3	Proximal Tubule Energy
Normal	Site of reabsorption of amino acids, glucose, bicarbonate; lacks peroxisomes (87)		Peroxisomes (87)	Proximal tubule ATP > distal tubule energy consumption (156); Glycolysis (neonate) → oxidative metabolism (adult) (35)
Response to stress (AKI): Ischemia/reperfusion; metabolic injury; hypoxia; toxins; sepsis	Autophagy, apoptosis → ATG	Fanconi syndrome; "sensor" of stressors in AKI: signals S2 and S3 responses (39); ↑ sirtuin and HO-1 (antioxidants) (87); ↑ urinary retinol binding protein (34)	↑ TGF-β; apoptosis, necrosis (39)	↓ oxidative metabolism; ↑ IF1 (endogenous inhibitor of mitochondrial ATPase) (74)
Regenerative response	Tubular progenitor cells seal urinary pole →ATG (48)	Cytoresistance to subsequent insults (194); cells in G1 phase ready to proliferate in response to stress (179); regeneration capacity is limited: AKI → proximal tubule shortening (162)		

Nos. in parentheses, reference nos. AKI, acute kidney injury; ATG, atubular glomeruli; HO-1, heme oxygenase-1; IF1, ATPase inhibitory factor 1; TGF-β, transforming growth factor-β.

and S3 segment necrosis following cisplatin or ischemia-reperfusion injury (100, 101).

Following ischemia-reperfusion injury, KIM-1 is upregulated by proximal tubule cells, which assume a macrophage-like phagocytic phenotype and engulf apoptotic and necrotic cells, favoring tubular repair (81). However, sustained proximal tubular expression of KIM-1 in transgenic mice (in the absence of injury) results in secretion of monocyte chemotactic protein-1, macrophage recruitment, and ultimately leads to interstitial fibrosis (80). In contrast to UUO and ischemia-reperfusion, sepsis-induced AKI is not characterized by tubule cell death, but rather by inflammation and oxidative stress that triggers an adaptive response of proximal tubule cells with downregulation of mitochondrial energy metabolism and cell cycle arrest (Table 2) (65).

Proximal tubule susceptibility to injury increases with maturation. The phenotypic response of the proximal tubule to injury is molded by the stage of kidney development or aging. Thus it should not be surprising that altered proximal tubular structure and function following obstructive, ischemic, toxic, or metabolic injury during nephrogenesis can differ from those in the mature or the senescent kidney. Either intrinsic renal disease (e.g., PKD, congenital nephrotic syndrome, or nephropathic cystinosis) or reduced nephron number at birth (due to prematurity or intrauterine growth restriction) increases susceptibility to episodes of AKI. The proximal tubule serves as sensor of the insult and as effector of responses for both AKI and CKD (Table 2).

Compared with distal tubules, proximal tubules are more susceptible to ischemic and toxic injury, largely a consequence of their lower capacity for anaerobic glycolytic ATP production and lack of antioxidant and antiapoptotic proteins (Table 2) (63, 89). However, factors secreted by distal tubules can signal proximal tubules through cross talk, thereby enhancing their survival (63). In addition, the proximal tubule is particularly vulnerable to mitochondrial toxicity: the relative activity of ATPase inhibitory factor 1, an endogenous inhibitor of the F1 subunit of mitochondrial ATP synthase, is greater in proximal than distal tubules, and production of reactive oxygen species is greater in proximal tubules (Table 2) (74). In rats 3–6 h following the release of UUO, proximal tubule mito-

chondria are swollen and vacuolated, and renal ATP production is decreased by 50% (11). Whereas adult proximal tubules have low levels of glycolytic metabolism (7), these levels are fourfold higher in the newborn than the adult rat (Table 2) (35), and mitochondria increase in size and number throughout the first 2 wk of life in the mouse (31). This would account for the relative resistance of the proximal tubule to injury following complete UUO in the newborn mouse: unlike the adult (48, 50), proximal tubule mitochondrial metabolism and glomerulotubular integrity are maintained for 14 days before undergoing mitochondrial loss, cell death, and formation of atubular glomeruli (49).

Energy metabolism. There may be a threshold of injury that activates the switch between recovery and glomerulotubular disconnection: patients with severe renal artery stenosis develop widespread formation of atubular glomeruli (Table 1) (110). Experimental renal artery stenosis in the rat treated with an angiotensin-converting enzyme inhibitor leads to severe renal ischemia and reduced kidney (proximal tubule) size after 7 wk (73). This is accompanied by a 97% reduction in GFR, atrophic (but not necrotic) tubules with reduced numbers of mitochondria, and 90% reduction in proximal tubule Na-K-ATPase: all of these changes were reversible within 3 wk following removal of the clip and withdrawal of enalapril (72, 73). This study reveals a remarkable adaptation by the proximal tubule to chronic ischemia by downregulation of proximal tubular mass, energy production, and reabsorptive function in concert with decreased GFR, a phenomenon the authors named "renal hibernation." Complete interruption of renal blood flow, a consequence of renal transplantation, may inflict a more severe tubular insult. Experimental renal transplantation induces mitochondrial uncoupling via uncoupling protein-2, increased kidney oxygen consumption, and decreased oxygen tension before tubular injury is detectable; diabetes results in similar mitochondrial uncoupling (Table 1) (52, 134). These early tubular responses may set the stage for diabetic or chronic allograft nephropathy, which lead to the formation of atubular glomeruli (116, 133, 186).

Urinary tract obstruction also leads to renal ischemia, and partial or complete UUO in the dog reduces renal oxygen consumption by 60%, with a doubling of the respiratory

quotient, consistent with a shift to glycolytic metabolism (Table 1) (158). Whereas either partial or complete UUO in weanling rats impairs mitochondrial oxidative metabolism, urinary hippurate and dimethylglycine excretion is increased following partial UUO, but decreased with complete UUO (106). As in renal artery stenosis, downregulation of proximal tubular oxidative metabolism is an adaptive response to UUO, and increased excretion of osmolytes (hippurate and dimethylglycine) may compensate for oxidative stress following partial UUO, a response that is abrogated by the severity of complete UUO (106). Nondiabetic patients with moderate CKD manifest decreased renal oxygen consumption, whereas diabetic patients with equivalent GFR have even lower renal oxygen consumption (94). Interestingly, following renal ablation, oxygen consumption by surviving nephrons more than doubles, but this does not result in oxidative stress unless animals are fed a high-protein diet (Table 1) (121). Taken together, these studies indicate that the proximal tubule can downregulate energy metabolism in response to diffuse chronic renal injury, but upregulate metabolism in intact nephrons in response to renal ablation. In the context of energy conservation, persistent upregulation of oxygen consumption by hypertrophied proximal tubules may not be sustainable, thereby contributing to the progression of CKD or aging.

Ischemic renal injury reduces mitochondrial number in the proximal tubule, which produces 14 μmol ATP/min (distal tubule, 6 μmol/min; remainder of nephron, <1 μmol/min) (156). Proximal tubular ATP sodium transport and gluconeogenesis compete for the same pool of ATP when supply is limited by ischemia (156). Adaptive energy conservation by the proximal tubule has also been demonstrated for AKI resulting from sepsis, which causes hypoxia and oxidative stress (65). In this setting, there is downregulation of mitochondrial metabolism resulting in maintenance of the cell membrane potential and cell cycle arrest at the G1/S checkpoint: this prevents the cell from undertaking a process that would lead to a lethal energy imbalance and cell death (65). Damaged mitochondria are removed by mitophagy, and energy is redirected to essential processes, such as maintenance of ion gradients (65). Thus proximal tubular function is temporarily killed in the benefit of individual cell survival. This is important because even sublethal mitochondrial injury can lead to the generation of reactive oxygen species, inducing irreversible injury and cell death (164). In cultured mouse proximal tubule cells, ATP depletion between 25 and 70% of control results in apoptosis, whereas ATP depletion below 15% leads to necrosis (102). As noted above, mitoquinone, an antioxidant targeted to mitochondria, can significantly delay progressive proximal tubular injury in a mouse model of nephropathic cystinosis (Table 1) (55). The importance of mitochondria in the pathogenesis of kidney disease is underscored by the case of a child with CKD and partial Fanconi syndrome. Renal biopsy revealed tubular atrophy with dysmorphic mitochondria, and focal tubular absence of cytochrome-c oxidase (a respiratory chain enzyme partially encoded by mitochondrial DNA), but preservation of succinate dehydrogenase, which is entirely encoded by nuclear DNA (160).

The kidney constitutes 1% of body weight but utilizes 10% of total body oxygen consumption, primarily devoted to proximal tubule sodium reabsorption (32). As pointed out by Homer Smith in his memorable book *From Fish to Philoso-pher* (155), the necessity for reabsorption of 180 liters of glomerular filtrate per day is the result of our evolutionary heritage. Transition from a marine to freshwater environment gave rise to a high filtering glomerulus that was necessary to counteract the osmotic gradient, but adaptation to land and homeothermy required reclamation of 99% of the filtrate, leading to the large energy requirement (69). The formation of atubular glomeruli in CKD may represent an atavism that harks back to a common ancestor with fish that were faced with adaptation from freshwater back to saltwater: aglomerular kidneys evolved independently on three occasions, each separated by about 50 million years (10). The daddy sculpin belongs to one of these groups, and microdissection of kidneys from this fish revealed that, with increasing age, nephrons undergo progressive degeneration of the glomerulotubular junction, resulting in atubular glomeruli and aglomerular tubules (67). It is likely that, with progression of CKD, microenvironments created by proximal tubule responses to injury (e.g., production of cytokines, growth factors, reactive oxygen species) activate this program.

Repair and Regeneration

Homeostasis or failed repair? Both AKI and CKD are characterized by a heterogeneous population of nephrons: those that undergo regeneration, and those that become atrophied and nonfunctional (86). Current understanding of progression from AKI to CKD is focused on signaling that promotes tubular atrophy, fibroblast proliferation, and collagen deposition, a result of "failed differentiation" of regenerating tubules (99). This has been ascribed to pathological arrest of tubule cells at the G2/M phase in a population of nephrons that is responsible for production of cytokines that stimulate surrounding interstitial inflammatory infiltration and fibroblast proliferation (178). A potential functional role for the proliferation of α-smooth muscle actin-positive myofibroblasts in the interstitium has been demonstrated following complete UUO in the rabbit. On exposure to norepinephrine or angiotensin II, strips of fresh renal cortex from the 8-day obstructed kidney show marked contractility compared with the intact opposite kidney; this response is attenuated in kidneys obstructed for 32 days (115). Peritubular contractile cells would oppose tubular dilation resulting from increased hydrostatic pressure, a response that decreases with decreasing intratubular hydrostatic pressure and increasing collagen deposition in later stages of obstructive nephropathy.

It appears that many differentiated proximal tubule cells are in the G1 phase of the cell cycle and can respond to injury by proliferating and initiating tubular repair (Table 2) (179). Proximal tubule cells that respond to injury by dedifferentiating and proliferating are CD24-positive cells scattered throughout the tubule (153). A novel transgenic mouse strain with timed targeted injury to proximal tubule cells develops massive proliferation of resident proximal tubule cells, but repair is incomplete, resulting in measurable shortening of the proximal tubules associated with interstitial fibrosis (41). This study reveals that there are limits to regeneration and predicts that repeated episodes of proximal tubular injury would result in irreversible CKD, as demonstrated in a separate study, described below (162). Nonetheless, the proximal tubule has developed cytoresistance (inherent in the tubule cells) that

increases its resistance to future superimposed episodes of AKI, including uremia-associated compounds that accumulate with renal insufficiency and can exert renoprotective effects (194).

These responses likely result from epigenetic remodeling of proinflammatory and profibrotic genes, such that a "maladaptive" response can develop with an imbalance of cytoprotective defenses (Table 2) (194). Enhanced protein acetylation by inhibition of histone deacetylases (HDAC) impairs epigenetic regulation of recovery from AKI. Inhibition of class I HDAC in mouse models of AKI aggravates renal injury and impairs renal regeneration with suppressed EGF receptor phosphorylation (165). Following ischemia-reperfusion AKI in the mouse, proximal tubule histone acetylation is transiently reduced, accompanied by downregulation of HDAC5 and upregulation of bone morphogenetic protein-7 in the regenerative response (112). Targeted deletion in proximal tubules of dicer, a key enzyme for micro-RNA (miRNA) production, markedly reduces tubule apoptosis and improves renal function and survival following ischemia-reperfusion AKI (183).

The regenerative capacity of the proximal tubule exceeds that of the distal nephron or glomerulus, and there is substantial interest in understanding the biology of renal progenitors (140). Following ischemic AKI, surviving intrinsic tubule epithelial cells are primarily responsible for tubular regeneration (79). Blockade of CXCR4-stromal cell-derived factor-1 reduces a surge in renal stem cells following UUO and impairs parenchyma regeneration after release of obstruction (135). The presence of adult kidney stem cells in the renal papilla may result from a favorable hyperosmolar and hypoxic microenvironment (131). It is tempting to postulate that the destruction of the renal papilla resulting from prolonged complete UUO contributes to irreversible parenchymal damage in this model. There is also growing evidence for the presence of progenitor cells lining Bowman's capsule. Epithelial cells lining the vascular pole of Bowman's capsule have the capacity to differentiate into podocytes, whereas cells lining the urinary pole can differentiate into tubule cells (141). These findings have implications for the process of glomerulotubular disconnection following UUO, and the growth of epithelial cells around the urinary pole of Bowman's capsule, maintaining the integrity of the capsule of the atubular glomerulus (48). The epithelial cells extending across the glomerulotubular junction are flattened and express mesenchymal markers (vimentin and α-smooth muscle actin) (see Fig. 4 in Ref. 48).

Fibrosis as limited repair. Lineage tracing suggested that proximal tubule cells expressing mesenchymal markers following UUO could undergo "epithelial-mesenchymal transition," crossing the basement membrane into the interstitium to become transformed into myofibroblasts (83). However, this finding could not be confirmed in subsequent studies, which suggest instead that myofibroblasts derive from resident interstitial fibroblasts or from pericytes (71, 77). While the proximal tubule can clearly promote contiguous interstitial collagen deposition through paracrine signaling (90), migration of transformed epithelial cells remains in doubt. Contrary to prevailing opinion, extracellular matrix deposition around injured tubules has not been proven to be detrimental, but more likely represents a self-limiting repair process that restricts injury, rather than damaging surrounding intact nephrons (86, 178). Notably, peritubular collagen deposition is reversed following recovery

from direct proximal tubular injury (162), or following early release of partial ureteral obstruction (150). Treatment of fibrotic pathways with c-ABL tyrosine kinase inhibitors carries the danger of also inhibiting pathways that are renoprotective and contribute to renal repair (172).

The Key Question: Is Proximal Tubular Injury Sufficient to Explain AKI-CKD Progression?

Data reviewed to this point support the temporal primacy of proximal tubular injury, with interstitial fibrosis as a later reparatory response. What is the evidence for proximal tubular injury as the sole initiator of AKI-CKD progression? In vitro studies have demonstrated that human proximal tubule cells exposed to cisplatin can convert fibroblasts to activated myofibroblasts (113). Selective depletion of mouse S3 segment of proximal tubule results in azotemia and albuminuria with polyuria progressing to anuria (147). Histological examination revealed S3 segment tubule cells exfoliated in the tubular lumen (147). Proximal tubule TGF-β is upregulated in AKI and CKD, which contributes to tubular cell injury, cell death, and recruitment of peritubular inflammatory cells with deposition of extracellular matrix (59). Following UUO, proximal tubule TGF-β is selectively upregulated compared with remaining tubule segments (53), and inhibition of TGF-β markedly reduces proximal tubular cell death in the obstructed kidney of adult mice (57). Transgenic mouse overexpression of proximal tubule TGF-β induces proliferation of peritubular cells and deposition of collagen: sustained expression of TGF-β leads to focal degeneration of nephrons, leaving atubular glomeruli (90). These studies show clearly that either direct or secondary injury to proximal tubule cells can recapitulate many of the features of AKI and CKD. Sublethal injury to S1 and S2 proximal tubule segments in transgenic mice results in acute cellular injury followed by inflammatory infiltration and tubular cell proliferation leading to recovery (70). In contrast, three insults at 1-wk intervals leads to interstitial capillary loss, fibrosis, and glomerulosclerosis (70). In a subsequent study using a similar transgenic model, α-smooth muscle actin-positive myofibroblasts emerged around KIM-1-positive injured proximal tubule cells, with formation of glomerulosclerosis and atubular glomeruli (Table 2) (162). When cocultured with fibroblasts, injured tubule cells from the transgenic mice stimulated production of extracellular matrix (162). Based on tissue-specific molecular targeting, these experiments strongly support the conclusion that proximal tubular injury alone can lead to the cascade of events, resulting in peritubular inflammation, interstitial fibrosis, and glomerulosclerosis. The dose-dependent effect of repeated proximal tubular injury also confirms that repeated episodes of AKI result in CKD (70, 162).

New Directions: Proximal Tubular Structure and Function

The anatomy of the kidney is highly complex, and multiple factors are ultimately involved in the progression of kidney disease, including glomerular and microvascular damage, as well as tubular and interstitial damage (145, 146). The clinical challenge is to identify early risk factors for susceptibility to kidney injury, and this review argues for the proximal tubule as a primary source for biomarkers of

AKI and CKD. The proximal tubule has multiple functional roles, and impairment of transport functions can result in Fanconi syndrome or selective transport disorders, as well as AKI and CKD (118). In this regard, proximal tubular dysfunction reflected by increased urinary excretion of retinol binding protein is an early predictor of a loss of renal allograft function, preceding structural nephron damage (Table 2) (34). A consensus conference was recently convened to identify promising pathways of kidney repair after AKI and concluded that restoration of structure should be considered in parallel with recovery of function (78). Novel targets in epithelial cells include mitochondrial biogenesis and function, cellular senescence/cell cycle, and miRNAs (3, 78). Deep sequencing in microdissected rat renal tubules has revealed segment-specific transcriptomes that link structure to function (98). This nonbiased approach will allow detection of proteins encoded by proximal tubules at earlier time points and is more specific than markers of inflammation or fibrosis (1, 92). Combining this information with the recently reported generation of kidney organoids from human-induced pluripotent stem cells promises a new era of human disease modeling for screening nephrotoxic drugs (many of which target the proximal tubule) or elucidating monogenic kidney diseases (such as nephropathic cystinosis) (137, 161). Mapping of gene expression in the developing kidney will further enhance interpretation of this data (17). The generation of proximal tubule-like cells from human-induced pluripotent stem cells has been used to evaluate nephrotoxicity of 30 compounds (88). Proximal tubule toxicity in humans could be predicted with 99.8% training accuracy and 87.0% test accuracy and revealed cellular mechanisms of injury were in agreement with animal and human data (88).

As terrestrial homeotherms, mammals evolved energetically expensive nephrons that are highly susceptible to hypoxia and oxidative injury; this "tradeoff" can impair survival and reduce reproductive success. Interpretation of the function of gene regulatory networks will require innovative use of model organisms to understand their role from embryonic life through senescence, as well as responses to environmental changes (61). This approach could explain some of the unique adaptations by mammalian kidneys to organismal life cycle and metabolism. Vampire bats and shrews have a very high protein intake, but do not have an increase in GFR commensurate with the urea excretory load: although azotemic, neither species develops hyperfiltration or CKD (152). In contrast, the hibernating bear undergoes continued urea production, but, through unknown metabolic processes, it can reutilize urea nitrogen for protein synthesis (152). The selection of model species should target mechanisms (conserved transcription factor modules and signaling networks) in independent evolutionary lineages (173), and the appropriate ecological context of the organism must be considered when determining a putative adaptation in the laboratory (60, 157). Some miRNAs are encoded in introns in the Hox gene locus, and some are 100% conserved throughout bilaterian evolution. It is likely that targets of miRNAs maintain constraints and trigger evolution: there has been continual addition of lineage-specific miRNA families throughout metazoan evolution (accrual of miRNAs correlates with increased morphological complexity) (91). On the one hand, miR-127 protects tubule cells from ischemic injury,

modulating its targets HIF-1α and kinesin family member 3B (78). By contrast, upregulation of proximal tubule miR-21 aggravates ischemic or obstructive injury by silencing PPAR-α-mediated peroxisome metabolism of fatty acids and silencing genes that inhibit reactive oxygen species generation in mitochondria (66). Chronic UUO in the mouse increases mainly distal tubule miR-21, associated with development of interstitial fibrosis, which is attenuated by blocking miR-21 (195). Following ischemia or UUO in miRNA-21 knockout mice, de-repression of redox metabolic pathways and PPAR-α lipid metabolism pathways is associated with decreased injury (21). Notably, although miR-21 engages target genes in interstitial cells in cardiac disease (169), the tubule epithelial cell is the initial target of miR-21 engagement in the kidney, with subsequent activation of interstitial fibrosis (21).

The life course progression of CKD has been emphasized throughout this review and illustrated in mouse models studied from birth to adulthood (obstructive nephropathy, PKD, and nephropathic cystinosis) (55, 56, 150). This takes into account the now well-established effects of fetal and neonatal environmental factors that are determinants of adult health and disease (62). Based on the hypothesis that urine of preterm neonates born before the completion of nephrogenesis would be rich in stem/progenitor cells, urine was collected from 1-day-old infants born at 31–36 wk gestational age. Cells were characterized for gene expression analysis and flow cytometry and immunofluorescence for protein expression analysis. Isolated cells expressed markers of nephron and stromal progenitors and protected cocultured tubule cells from cisplatin-induced apoptosis (4). This previously untapped source of progenitor cells holds significant promise for kidney regenerative therapies.

The Kidney Research National Dialogue, supported by the National Institute of Diabetes and Digestive and Kidney Diseases, has placed particular emphasis on targeting the early stages of CKD (12, 103). The selection of optimal animal models is equally important to study the loss of homeostasis with aging (103). Senescence reflects a gradual cumulative process not modeled by acute severe injury, such as temporary complete interruption of renal blood flow (a model of AKI), or complete UUO (a model of CKD): new models should more closely parallel clinical disorders. Shifting attention from established interstitial fibrotic processes to early responses by the proximal tubule would significantly expand future research initiatives in the study of progressive kidney disease.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.L.C. conception and design of research; R.L.C. performed experiments; R.L.C. analyzed data; R.L.C. interpreted results of experiments; R.L.C. prepared figures; R.L.C. drafted manuscript; R.L.C. edited and revised manuscript; R.L.C. approved final version of manuscript.

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