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Transcription Factors in Kidney Development and Renal Injury

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INTRODUCTION

The kidney is a very heterogeneous organ with many types of cells having different phenotypic characteristics. This complexity can be traced, at least in part, to the differential activation of various genes, resulting in the expression of some proteins primarily in the kidney. The expression of kidney-specific proteins may be determined either by kidney-specific transcription factors or by transcription factors that are expressed in more than one organ but whose coordinated expression may be unique to the kidney. Examples of proteins that are expressed predominantly or exclusively in the kidney include the V2 vasopressin receptor (1,2) a chloride channel (3), and a Na/P_i cotransporter (4). Other proteins, though not specific to the kidney, are expressed during specific times in renal development and repair and contribute to the renal phenotype. Many transcriptional regulatory proteins are synthesized in a limited number of cell types. Kid-1 (see below) is an example of such a protein whose expression occurs predominantly in the kidney (5), as is HFH-3, a member of the hepatocyte nuclear factor 3/forkhead DNA-binding-domain family (6). Transcription factors are essential for the control of renal development and differentiation. In addition, they are likely important for the regulation of processes involved in repair of the kidney after an injury. Kidney injury and repair recapitulate many aspects of development, since they involve dedifferentiation and regeneration of epithelial cells followed by differentiation (7–10). The ability to regenerate epithelial structure and restore differentiated function after an ischemic or toxic insult is an important property of the kidney, an ability not possessed by heart and brain, where myocytes or neurons cannot be replaced.

TRANSCRIPTION

The regulation of transcription in eukaryotes is much more complex and less well understood than the relatively simple switch mechanisms of prokaryotes (11). An initial step in the activation of any gene is the interaction of a set of proteins (*trans*-acting elements, or transcription factors) with "regulatory" elements in the gene (*cis*-acting elements), resulting in the initiation of a sequence of events that leads to an increase in the cellular levels of mRNA and ultimately the protein encoded by that gene (Figure 1). The proteins involved in this process must recognize DNA or recognize other proteins in a regulatory region of DNA. Interactions between these proteins and the DNA can occur within a few hundred base pairs or much further away from the site in the gene where transcription starts (the "transcriptional start site"). The more proximal region upstream of the start site is termed the "promoter" region. More distal *cis*-acting elements are referred to as "enhancers." The promoter region of most genes contains a region rich in A-T base pairs, the so-called TATA

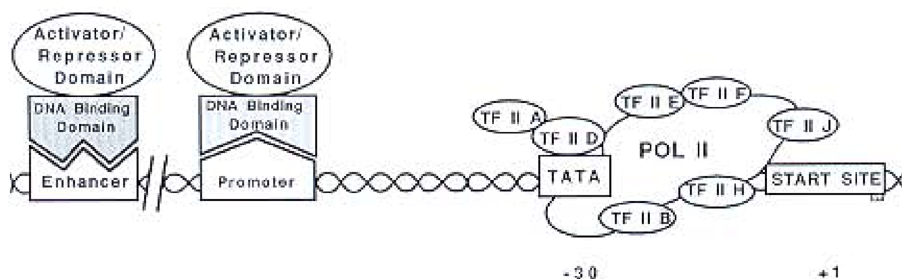

Figure 1 *Cis*- and *trans*-acting elements in the regulatory region of a gene. DNA binding proteins (the *trans*-acting components in the regulatory machinery) typically possess a DNA-binding domain and a *trans*-acting domain, which can have activator or repressor function or both. The specific DNA-binding proteins bind at particular sites in the regulatory region of the gene, the *cis*-acting elements, which can be very close ("promoter") or at a far distance ("enhancer") from the transcriptional start site. The transcriptional start site is defined as the nucleotide in the DNA that is complementary to the first nucleotide in the mRNA. After the specific transcription factors have bound to the DNA, they interact with the basal transcription factors (TFIIA, B, D, E, F, H, J) and promote their assembly together with RNA poymerase II over the TATA box. The TATA box (typically a nucleotide sequence of "TATAAA" or "TATATA") is recognized by the "TATA binding protein (TBP)," which together with the "TBP-associated factors (TAFs)" constitutes TFIID. Subsequent to the binding of TFIID, the other basal transcription factors and RNA polymerase II assemble and transcription can begin.

box, located approximately 30 bp upstream from the transcriptional start site. Transcription factors that activate gene expression, and some that serve as repressors, have two functionally distinct domains: a DNA binding domain and a *trans*-acting domain (11). Protein-DNA interactions occur via the DNA binding domain, which positions the protein on the DNA so that the *trans*-acting part of the protein can interact with other proteins such as those being assembled at the site of the TATA box (the "basal transcription factors"). The fact that DNA can fold allows for the interaction of a transcription factor that binds to DNA many kilobases upstream from the TATA box with proteins close to the site of initiation of transcription and hence allows for regulation from great distances. Other explanations for long-range interactions include the assembly of several proteins spanning the region between the distant *cis*-acting element and the transcriptional start site as well as alterations in the structural properties of the DNA.

Transcription factors can be classified into two groups. One group, general or basal transcription factors, is necessary and sufficient, at least in vitro, for transcription from a TATA box. The second group of transcription factors act only at certain promoters. It is this second type of transcription factor that is discussed in greater detail in this chapter.

STRUCTURAL MOTIFS OF EUCARYOTIC TRANSCRIPTION FACTORS

The DNA-binding regions of many eukaryotic transcription factors are built from a limited number of motifs. Many, but not all, transcription factors are members of three families: zinc-finger, leucine-zipper, and helix-turn-helix proteins. Whereas the zinc-finger and helix-turn-helix motifs are DNA-binding motifs, the leucine zipper is a domain involved in protein-protein interactions.

Zinc-Finger Proteins

The analysis of the amino acid sequence of *Xenopus laevis* TFIIIA, an abundant protein that regulates transcription of the gene for ribosomal 5S RNA, led Klug to postulate that the zinc-finger (Figures 2 and 3) represented a DNA-binding motif (12). TFIIIA has nine repeats of 30 amino acids each with two cysteine residues at one end and two histidine residues at the other. The last histidine

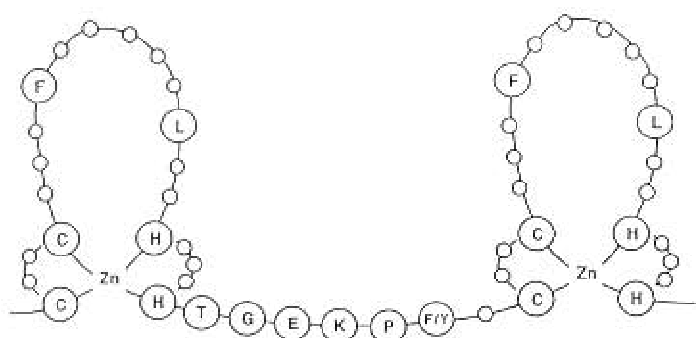

Figure 2 Schematic diagram of a zinc-finger protein of the C_2H_2 family. The members of the C_2H_2 family of zinc-finger proteins are characterized by two cysteines and two histidines, which coordinate a zinc ion at the base of a finger-like structure ("zinc finger"). "F" (phenylalanine) and "L" (leucine) are highly conserved residues in many of these proteins. The zinc finger itself constitutes the DNA-binding domain. Between the individual finger domains, immediately following the second histidine, a highly conserved structure can be found. This so-called "H/C-link" has the sequence "TGEKP(Y or F)." The function of this linker region is not fully understood.

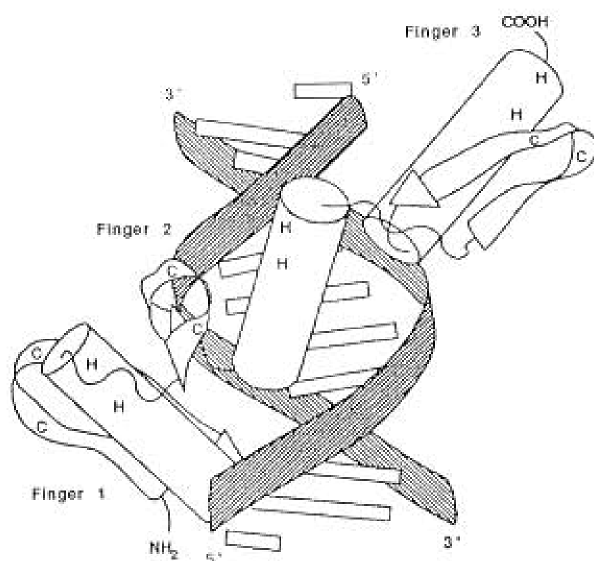

Figure 3 One model for the recognition of DNA by C_2H_2 zinc-finger proteins. The structure of a C_2H_2 zinc-finger/DNA complex was solved for the first time by the analysis of a cocrystal between the three zinc fingers of zif268/Egr-1 and their recognition sequence (92). A model was derived in which each individual finger is folded in a very similar fashion. The NH_2-terminal part of a finger consists of a two-stranded antiparallel beta sheet (depicted as ribbons) and is followed by an alpha helix (shown as a cylinder). The approximate positions of the two cysteines and two histidines in each zinc finger are shown. In the case of zif268/Egr-1, amino acids in all three zinc fingers make contacts in the major groove of the DNA. Analyses of two other crystal structures, however, indicate that alternative models are required to explain the interaction between C_2H_2 zinc fingers and DNA. (From Ref. 92.)

residue and the first cysteine residue of the next repeat are often separated by a highly conserved region of 12 amino acids. Since the protein contained zinc and the zinc was critical to the transcriptional control function of the protein, Klug proposed that the cysteines and histidines were complexed together by zinc and that the intervening amino acids then formed a finger-like structure which would fit into the major groove of the DNA double helix.

Since the original description of this motif, a very large number of proteins containing it have been identified. The number of repeats varies a great deal among this group of proteins. These Cys_2-His_2 proteins have been joined by other families of zinc-finger proteins, such as the Cys_2-Cys_2 family and the Cys-X_2-Cys-X_6-Cys-X_6-Cys-X_2-Cys-X_6-Cys family (where X is any amino acid). The glucocorticoid, thyroid hormone, retinoic acid, vitamin D_3, progesterone, estrogen, and mineralocorticoid receptors belong to the Cys_2-Cys_2 family. GAL 4 is an example of a member of the third zinc-finger family. This protein from *Saccharomyces cerevisiae* is necessary for transcription of genes encoding galactose-metabolizing enzymes. In this family, it is proposed that the six cysteine residues are held together in a cluster by zinc.

Leucine-Zipper Proteins

The leucine zipper motif was first recognized in the yeast transcription factor GCN 4, which activates transcription of the *his*3 gene (13). This motif is also found in C/EBP, fos, jun, and myc. In each of these proteins there is a region of approximately 30 amino acids where each seventh amino acid is a leucine. The leucines form a ridge on one side of an α-helix, so that the leucines from two α-helices can interact with each other. The leucines are apposed to each other, rather than interacting in a zipper-like fashion as had been originally thought. The leucine zipper therefore represents a dimerization domain (Figure 4). Dimerization is necessary for transcription factor function. A number of proteins that have this structural motif, such as GCN 4, fos, and jun, have been shown to form homo- and heterodimers with this region mediating the dimerization. The DNA-binding region of the proteins is a basic domain amino terminal to the leucine zipper.

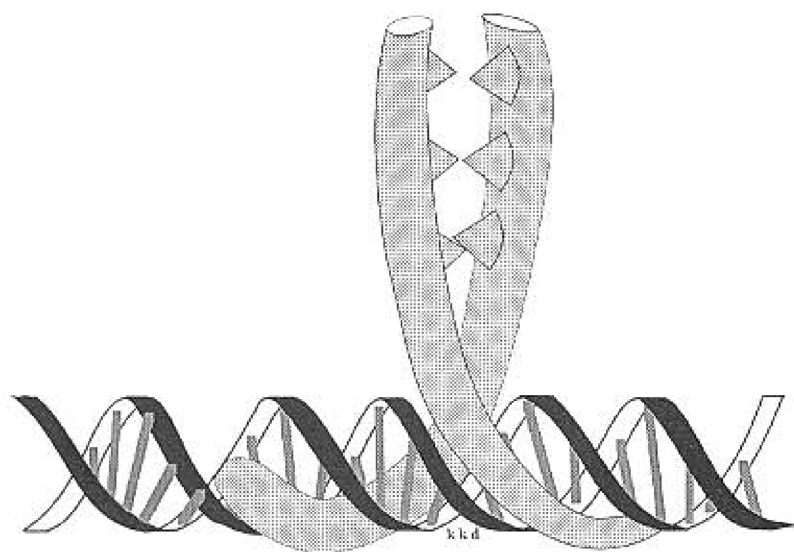

Figure 4 Leucine-zipper proteins bind to DNA as dimers. In leucine-zipper proteins, another common class of transcriptional regulatory factors, the heptad repeats of leucines (depicted as spikes in this schematic diagram) are arranged on one side of an alpha helix and serve as dimerization motifs. The term "leucine zipper" is misleading, because the leucine zipper domains from two proteins do not interdigitate but rather lie adjacent to each other. The basic domain in each protein contacts its recognition site by interacting with the DNA in the major groove. Efficient binding to the DNA requires bending of the protein and as a consequence the dimer assumes a Y-shaped structure. (Adapted from Ref. 94.)

Helix-Turn-Helix Proteins

This is a common motif in transcriptional activators and repressors in prokaryotes, where it was first described (14). Many of the proteins in this family regulate cell-specific transcription. The DNA-binding region consists of two α helices separated by a β turn. In eukaryotes the helix-turn-helix motif is used in "homeodomain" proteins. The homeodomain is a region of 60 amino acids, consisting of three α helices held together by short linker regions of amino acids. While prokaryotic helix-turn-helix proteins bind to DNA as dimers, eucaryotic homeodomain proteins bind DNA as monomers (Figure 5). These proteins are particularly important for eukaryotic development, since it has been found that the genes are tightly regulated during development and are capable of "homeotic" transformations where one structure is transformed into another (e.g., in the case of antennapedia, the transformation of antennae into an additional pair of legs).

Space constraints prevent a more detailed overview of the properties of eukaryotic transcriptional regulation. The reader is referred to various books and reviews that discuss the general aspects of the structure of transcription factors and what is known about how they interact with DNA (14–19).

RENAL DEVELOPMENT

Kidney development proceeds in three stages from the pronephros to the mesonephros and finally the metanephros. The first two stages are transitory but necessary stages in the ultimate development of the mammalian kidney, which is derived from the metanephros. At approximately the end of the second gestational week in the mouse and rat, the first steps of metanephrogenesis occur. The kidney is remarkable in that many of its epithelial structures (proximal tubules, thin limbs of Henle, and distal tubules) originate from mesenchymal precursors. The collecting duct system derives from the ureter, which invades the metanephric blastema mesenchymal cells and undergoes consecutive branching. The buds of the ureter branches interact in a very close fashion with the loose metanephrogenic mesenchyme, first resulting in an aggregation of the mesenchymal cells around

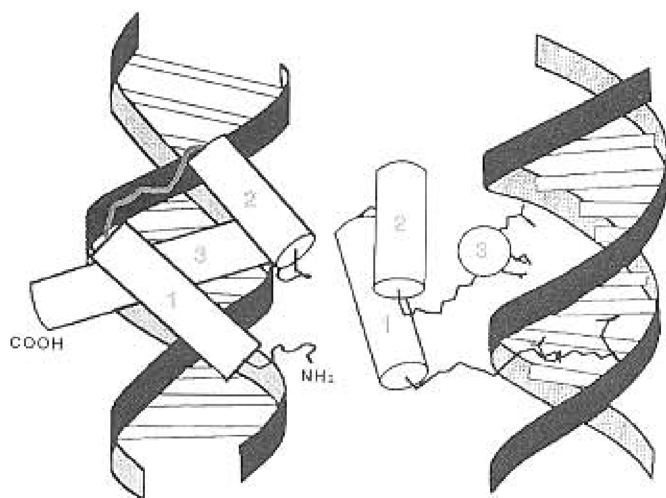

Figure 5 Interaction between a eukaryotic helix-turn-helix protein and DNA. The schematic diagram is derived from the structural analysis of a eukaryotic helix-turn-helix motif, in this case the homeodomain of the engrailed protein, and its recognition site (93). The right-hand side of the figure represents a 90° rotation of the diagram on the left. Helices 2 and 3 (shown as barrels) are separated by a beta turn and together form the conserved helix-turn-helix motif. Whereas in prokaryotes helix-turn-helix proteins bind to DNA as dimers, eukaryotic members of this family interact with DNA as monomers. Contact points are established by residues NH_2-terminal to the first helix, which reach into the minor groove of DNA, and by amino acids in the third helix, which make contact in the major groove. (From Ref. 93.)

the buds. The condensed mesenchyme undergoes several dramatic morphological changes into comma- and S-shaped bodies, a lumen forms in the cell mass, a basement membrane is established, and blood vessels grow into the proximal portion of the prospective nephron. The tubular structures will fuse at their distal ends with the collecting ducts. A continuous lumen develops, and a fully (but not terminally) differentiated nephron forms. In the rat, S-shaped bodies can be found until 4 to 5 days after birth (20), and thymidine incorporation, used as an indicator for cell proliferation, does not decline to baseline levels until 2 to 4 weeks after birth (21,22). By contrast, in humans, kidney development is complete by the time of birth. The morphological sequence of events that occurs in the developing kidney has been described in great detail. (For excellent reviews on this subject see, Refs. 23 and 24.)

Differentiation of the metanephric blastema cells into epithelial cells is a critical event in kidney morphogenesis. What makes an epithelial cell distinct from a mesenchymal cell has to lie to some extent in the induction of specific genes that will confer the phenotype. An approach to the understanding of this process is to know the genes that are induced with this transition from mesenchyme to epithelium and then trace back which *trans*-acting factors control the activation of those genes. Some of these *trans*-acting factors are likely to be "kidney-specific."

Which Genes Are Induced with Renal Development?

The dramatic morphological changes that occur as the kidney forms into a complex heterogenous structure have to be accompanied by similarly drastic changes in the genetic program of the differentiating cells. A basement membrane has to be formed, the apical surface of the newly formed epithelium has to be demarcated by different kinds of junctions, and a brush border will appear in the proximal tubule. The different tubular segments must be invested with different permeability and transport characteristics to electrolytes, water, and urea (25). In recent years evidence has accumulated for the activation and repression of certain genes that have been implicated in the transition from the mesenchymal precursor cells to the fully developed nephron. These genes encode proteins belonging to a wide variety of different families, such as extracellular matrix proteins, integral membrane proteins, cytoskeletal proteins, and nuclear proteins. Early in kidney development, cells express collagen I and III (26) as extracellular matrix proteins, N-CAM (27), syndecan (28,29), and α_1- and α_2-integrins (30) as cell surface proteins and vimentin as an intermediate filament-type cytoskeletal protein (31,32). In the differentiated tubular cells, those proteins will disappear and new proteins will emerge. The tubular epithelium now expresses collagen IV (26) and laminin A (33) as building blocks of the basement membrane; uvomorulin/E-cadherin (27); α_2-, α_3-, and α_6-integrins, which substitute for N-CAM; and α_1- and α_4-integrins (30). Vimentin is replaced by cytokeratins (31,32), and new enzymes [alkaline phosphatase, g-glutamyltransferase (21)]. Transporters such as the P-glycoprotein (34) and glucose transporters (35), and different junctional components [desmosomal as well as gap junction proteins (36,37)] are synthesized so that the renal epithelia can fulfill their characteristic functions.

The signals responsible for these changes in the genetic program have been poorly defined. TGF-α (38) and the receptor for IGF-I (39) have been shown to be necessary for metanephric development in vitro. Hepatocyte growth factor and its receptor, the c-met tyrosine kinase, are expressed transiently during metanephric development and may be of importance (40–42). (For a review on the role of growth factors in renal development see Ref. 43.) How changes in the genetic program(s) occur is not clear at present, because the *trans*-acting factors regulating the structural genes in general and how they are modulated during renal development in particular are largely unknown. This review attempts to describe different transcription factors and their potential role in the kidney. Among the genes we discuss are the *myc* family of protooncogenes, the paired box genes *Pax2* and *Pax8*, the genes encoding the homeodomain proteins LFB1 and LFB3, and the zinc finger genes *Kid-1* and *WT-1*. Other DNA-binding proteins of interest that will not be discussed further in this chapter are Hox-3.2, a homeodomain protein (44,45), and MFH-1 (46) and HFH-3 (6), two members of the HNF-3 family (also see below). Another protein that may be important for renal development but for which there is little information at present is δEF1. This transcription factor has both zinc fingers and a homeodomain. In the mesonephros δEF1 is expressed briefly. It is expressed again to a strong level in the condensed mesenchyme and early metanephric tubules, but tubule structures cease the expression of δEF1 after the tubule structures mature to the point where

they connect to the Wolffian duct (47). Since entire chapters in this book are devoted to the Pax family of transcription factors and WT-1, these proteins are discussed only briefly.

THE *myc* FAMILY OF PROTOONCOGENES

The first member of the *myc* family of nuclear protoonocogenes to be isolated was c-*myc*. It represents the cellular homologue of the transforming sequence in the avian myelocytomatosis virus (AMV). Subsequently, two other members were identified in neuroblastomas and small cell lung carcinomas, N-*myc* and L-*myc*, respectively. The products of the three full-length members of the *myc* gene family—c-*myc*, L-*myc*, and N-*myc*—are among the most thoroughly investigated nuclear protooncogenes (besides those three genes, two other truncated *myc* genes have been cloned, B-*myc* and S-*myc*). (For reviews, see Refs. 48 and 49.) They can be classified as basic helix-loop-helix/leucine-zipper proteins, where a basic domain functions as the DNA-binding portion and the helix-loop-helix and leucine-zipper domains serve as dimerization motifs mediating protein-protein interactions. The c-*myc* gene has been implicated in cell proliferation, whereas the roles played by other *myc* genes are less well defined (50). High levels of N-*myc* have been found in Wilm's tumor and other tumors of embryonic and neuroendocrine origin (51–53). By employing PCR, a binding site for c-*myc* has been defined; it is the so-called E box motif "CACGTG." Despite efforts from many laboratories, no clear-cut target gene for the Myc proteins has been found, and until recently no interacting proteins were cloned. Recently identified proteins that interact with c- and N-Myc include Max (54), Mxi1 (55), and Mad (56). Like Myc itself these proteins also contain basic helix-loop-helix/leucine-zipper motifs. Through in vivo and in vitro experiments, it could be shown that Max and Mad can form homodimers and heterodimers with each other and also with Myc (54,56,57), and that Mxi heterodimerizes with Max (55). Thus there are several possibilities for interactions among the different proteins. Each of the different complexes also differs in its DNA-binding and *trans*-acting activities (58). One can therefore imagine a very complex network with varying biological activities resulting from the various interactions among these four proteins, and it is likely that as yet unknown other proteins may interact with these. In interpreting the expression pattern of the different members of the *myc*-family, this fact has to be kept in mind.

The expression patterns of c-*myc*, L-*myc*, and N-*myc* during kidney development differ strikingly from each other. The c-*myc* mRNA can be found in all proliferating cells in the developing nephron, i.e., loosely aggregated and condensed mesenchymal cells, the comma- and S-shaped bodies, and in the elongating tubule. The c-*myc* gene is not expressed in the fully developed nephron (59,60). mRNA for L-*myc*, however, can only be detected in cells with a very low mitotic rate, that is, in the cells of the ureter stems in the early stages of the developing kidney. In the collecting ducts of the adult kidney, L-*myc* can no longer be found (59,60). The N-*myc* gene, the third member of the *myc*-family, is activated a little later in development than its cousin c-*myc*. N-*myc* appears first and at its highest levels in the condensed mesenchyme. Thereafter N-*myc* expression declines, so that the latest stage at which its mRNA is present is in the S-shaped bodies (59,60).

How do these developmental findings relate to findings obtained in pathophysiological states of the kidney, such as acute renal injury, polycystic kidneys, and renal tumors? In a model of folic acid–induced acute tubular necrosis in the mouse, elevated levels of c-*myc* could be detected at 24 hr after the intraperitoneal injection of folic acid, consistent with the notion that c-*myc* is expressed in the regenerating cells that enter the cell cycle (61). In the kidneys of mice [C57BL/6J (*cpk*)] homozygous for a hereditary form of autosomal recessive polycystic kidney disease, there were elevated levels of c-*myc* expression at 2 and 3 weeks after birth as compared to expression in kidneys from normal animals (61,62). c-*myc* mRNA has been localized to the lining epithelium of collecting duct cysts as well as normal-appearing proximal tubules of mice with polycystic kidney disease (62a).

More direct insight into the roles played by the *myc* family of transcription factors comes from the establishment of transgenic mice carrying a murine c-*myc* transgene under the control of the SV40 enhancer and mice in which the N-*myc* locus has been mutated by homologous recombination. The mice carrying the c-*myc* transgene consistently developed polycystic kidney disease and died of renal failure between 6 weeks to 3 months after birth (63). This supports the hypothesis that c-*myc* plays a role in the pathogenesis of polycystic kidney disease, though as yet there are no naturally

occurring mutations in any of the *myc* genes found in hereditary or spontaneous renal diseases in humans or animals. Homozygous mice with a mutated N-*myc* locus die prenatally at approximately 11.5 days of gestation (64). A number of developing organs are affected, including the mesonephros, nervous system, gut, and lung. The entire mesonephros is disorganized. Although some tubules appear to be normal, others fail to form or degenerate prematurely. There is also an abnormality of the genital ridge in these animals.

THE PAIRED BOX GENES *Pax2* AND *Pax8*

Pax2 and Pax8 belong to a subgroup of homeodomain proteins, the paired box family. This family contains another highly conserved structure in addition to the homeodomain, the paired box, which was first described in three *Drosophila* segmentation genes (65) and subsequently found in other species such as mammals, worms, fish, amphibia, and birds. Recently it was established that some members of the *Pax* family of proteins possess oncogenic potential (65a). (For a more in-depth description of the *Pax* genes, the reader is referred to Chapter 1 of this book and to recent reviews—Refs. 66 and 67.)

Pax2 and N-*myc* are very related in their expression patterns. In a manner similar to N-*myc* mRNA, *Pax2* mRNA and protein are expressed first and most highly in the condensed mesenchyme, after which their expression declines. The latest stage in which *Pax2*-positive, mesenchyme-derived cells are seen is in the "S-shaped body" stage. In striking contrast to N-*myc*, however, *Pax2* mRNA and protein are also present in the ureter buds and even beyond the S-shaped stage in the collecting ducts (68–70). In a recent report the establishment of transgenic *Pax2* mice has been described. The *Pax2* transgene was placed under the control of the cytomegalovirus (CMV) enhancer, a strong enhancer active in wide variety of different cell types. Despite the expression of the *Pax2* transgene in kidney, hindbrain, liver, lung, pancreas, heart, and gut, only kidney abnormalities and precocious opening of the eyes were reported. The anatomic abnormalities and laboratory parameters of the transgenic mice resemble features described for congenital nephrotic syndrome (71).

Like the *myc* genes, different members of the *Pax* family are expressed at different times during development. *Pax8*, like *Pax2*, appears early, its mRNA is detected first in the condensed mesenchyme. Thereafter, however, its expression increases to reach its peak at the S-shaped stage. Beyond the S-shaped bodies, *Pax8* mRNA is absent. This implies, that the *Pax8* gene is shut off abruptly and/or that the *Pax8* mRNA becomes increasingly unstable. Furthermore, at no time during renal development is *Pax8* present in the ureteral buds, thus differing from *Pax2* (72,73).

As yet, no genetic evidence exists that *Pax2* or *Pax8* are involved in the pathogenesis of spontaneous or hereditary renal disease, though overexpression of *Pax2* in transgenic mice leads to congenital nephrotic syndrome (71). Three other members of the *Pax* gene family have been implicated in hereditary disorders involving organs other than the kidneys. In the mouse, a mutant exists, called *undulated*, which is characterized by a kinky tail and vertebral malformations and is probably caused by a mutation in the *Pax1* gene (66,67). Mutations in the *Pax3* gene have been made responsible for two corresponding syndromes in mouse (*splotch*) and humans (Waardenburg syndrome) (66,67). The mouse *splotch* mutants present with abnormalities in the nervous system (exencephaly, spina bifida, meningocele, absence or reduction of dorsal root ganglia), white spotting, and heart defects. The Waardenburg syndrome in humans manifests itself as deafness, pigmentary deficiency (heterochromia irides, white forelock and eyelash), and in some cases a lateral displacement of the inner corner of the eye. Two very similar conditions are caused by mutations in the *Pax6* gene, *small eye* in the mouse and aniridia in humans (66,67). *Small eye* mice present with underdevelopment of the eye or even with complete absence of eyes and nose. Patients with aniridia show partial or complete absence of the iris, which is often accompanied by impaired vision, cataracts, and other eye defects.

LFB1 AND *LFB3*, TWO TRANSCRIPTION FACTORS INVOLVED IN THE ACTIVATION OF LIVER-SPECIFIC GENES

It is possible to learn a good deal about organ-specific gene activation by examining the genetic programs operant in the liver, where a number of proteins have been identified which are expressed

predominantly in this organ. The activation of these genes is not controlled by liver-specific transcription factors but by transcription factors which can be found in many different organs, such as LFB1 (HNF-1, HNF-1α), LFB3 (vHNF-1, HNF-1β), HNF-3, HNF-4, and CCAAT-enhancer binding protein (C/EBP). They contain very diverse sequence motifs found in different families of transcription factors, such as POU domains (HNF-1), basic leucine-zipper motifs (C/EBP), forkhead domains (HNF-3), and zinc fingers (HNF-4) (for reviews see Refs. 74 and 75).

At first, the fact that these transcription factors are not exclusively expressed in the liver appears very surprising. At second glance, however, this finding also makes sense, because employing organ-specific transcription factors to regulate organ-specific genes only postpones the problem of how to establish organ specificity. In the case of the liver, it is the interaction of more or less "promiscuous" transcription factors, which regulate the genes encoding organ-specific proteins such as albumin, α1-antitrypsin, and transthyretin. Two of these transcription factors, which are involved in the expression of liver-specific proteins, are also expressed in the context of renal development; they are LFB1 and LFB3.

The expression of *LFB3* occurs with time characteristics somewhat reminiscent of those of *Pax8*. Its mRNA is first detected in the condensed mesenchymal cells and reaches its peak in the S-shaped bodies. Whereas *Pax8* cannot be found beyond this stage, however, *LFB3* mRNA remains present in the maturing and fully differentiated tubules and collecting ducts, though at lower levels than in the S-shaped bodies. The expression of *LFB3* mRNA in the ureteric buds occurs with a pattern similar to that of *Pax2* and quite distinct from that of *Pax8* (76).

LFB1 mRNA appears much later than *LFB3* mRNA and is absent in the ureter buds. It can be seen for the first time in the S-shaped bodies and is present even in the fully developed tubules (76).

THE ZINC-FINGER GENES *Kid-1* AND *WT-1*

Of all DNA-binding proteins, the zinc-finger family of transcription factors has the largest number of known members. It is estimated that there are several hundred zinc-finger genes in the mammalian genome. Two representatives of the zinc-finger family are discussed: *WT-1* and *Kid-1*.

WT-1

The *WT-1* gene was identified by positional cloning techniques as the gene responsible for a hereditary form of pediatric kidney tumors—nephroblastoma or Wilms' tumor. The gene lies on human chromosome 11p13 and is composed of 10 exons (77). It belongs to the rapidly expanding family of tumor suppressor genes, the counterpart of protooncogenes. Most tumor suppressor genes are defined on a genetic level; that is, inactivating mutations in both alleles of a certain gene are required in order to render a cell malignant (Knudson's "two-hit hypothesis" of malignant transformation)—a mechanism very different from that of protooncogenes, where one activating mutation or high expression of the protein product will transform a cell. The WT-1 protein contains four zinc fingers at its COOH-terminus, the specificity of which makes WT-1, together with Sp1 and the Egr proteins (78,79), a member of a family of transcription factors that recognize GC-rich binding sites. Alternatively spliced forms of the *WT-1* transcript are known to occur. These forms differ in their DNA-binding characteristics. Through transfection assays, it was possible to determine that the WT-1 protein acts as a transcriptional repressor, whose presumptive target genes are *IGF-II* and *PDGF-A* and potentially many more. (For reviews on *WT-1*, see Refs. 80 and 81 and Chapter 52 of this book.)

The *WT-1* transcript, like many of the other transcription factors mentioned above, appears first in the condensed mesenchyme. In the comma-shaped bodies, the steady-state level of the *WT-1* mRNA increases. At the next stage, in the S-shaped bodies, *WT-1*–expressing cells are still present. They are, however, not distributed evenly throughout the S-shaped bodies but are concentrated at the proximal end. This pattern anticipates the localization of *WT-1* in the adult kidney, where *WT-1* is expressed only in the podocytes of the glomerulus (83–85). The importance of *WT-1* is emphasized by the fact that mice in which both alleles of *WT-1* have been deleted (so-called knockout mice), no kidney development occurs. The differentiation of the metanephros in the knockout mice stops at an early stage. The ureter does not invade the metanephrogenic mesenchyme and does not branch (86).

Kid-1

In an attempt to find other transcription factors which play a role in kidney development and repair after renal injury, we screened cDNA libraries from normal and postischemic adult rat kidneys with an oligonucleotide encoding a consensus sequence present in zinc-finger proteins. It was possible to clone a novel cDNA, *Kid-1* (a gene expressed predominantly in the kidney and regulated in ischemia and renal development). *Kid-1* codes for a 66-kDa protein with 13 zinc fingers. These zinc fingers are clustered in groups of four and nine fingers and lie at the COOH-terminus of the protein. The mRNA as well as the protein contain instability motifs, suggesting a short half-life of both mRNA and protein. The protein also contains a nuclear targeting signal. At the very NH_2-terminus of the Kid-1 protein lie two highly conserved motifs, the KRAB-A and KRAB-B domains (5).

By Northern blot analysis and reverse transcriptase PCR, *Kid-1* can only be detected predominantly in the kidney. As already suggested by its acronym, *Kid-1* is regulated during renal development. As mentioned in the introduction to this review, the rat kidney has not fully matured in the newborn animal. At time of birth, the steady-state level of *Kid-1* is very low. After birth, the expression of *Kid-1* slowly increases and reaches the highest levels in the adult animal (5).

There is increasing evidence that recovery after renal injury and renal development are related in a reverse manner. In the injured organ, necrotic and apoptotic cells have to be replaced by the surviving epithelial cells. This can only be achieved if the latter leave G_0-phase and enter the cell cycle. At about the same time, the cells dedifferentiate. They now express proteins that are only present early in development or they shut off genes that are only activated late in development. Several models of acute tubular necrosis exist, such as temporary interruption of blood flow by occlusion of the renal artery and intraperitoneal injection of high concentrations of folic acid. Within 5 hr of reperfusion after renal ischemia, *Kid-1* mRNA disappears. It takes approximately 7 days after ischemia for the *Kid-1* mRNA levels in the post-ischemic kidney to return to normal. Very similar results were obtained by intraperitoneal administration of folic acid. By 3 hr after injection of folic acid, *Kid-1* mRNA levels start to decline (5).

The above findings suggest that the expression of *Kid-1* is low in cells that proliferate and are not fully differentiated. An important example of continuously dividing cells in a low-differentiation state is cancer. In the rat, a hereditary form of renal cell carcinoma has been described—the Eker tumors (87). When RNA from three samples of Eker tumors was examined, the *Kid-1* transcript was present at lower levels than in the adult normal kidney (unpublished data). Similarly, in epithelial cell lines from the rat kidney, *Kid-1* mRNA was barely detectable (unpublished results).

In mouse, rat, and human *Kid-1* is present as a single-copy gene. By linkage analysis, somatic hybrid experiments, and fluorescence in situ-hybridization, the *Kid-1* gene was localized to rat chromosome 10q21.3-q22, possibly corresponding to mouse chromosome 11 and human chromosome 5. The rat *Kid-1* gene extends over approximately 14 kbp. The protein coding region is divided into four exons and three introns, closely mirroring the domain structure of the protein. The first exon contains the 5′-untranslated region and sequence coding for the first 11 amino acids. The second exon encodes only and entirely the KRAB-A domain. The largest part of the third exon is composed of KRAB-B coding sequence. All 13 zinc fingers are encoded by exon 4 (88) (Figure 6). We recently have identified an additional 5′ exon that encodes nontranslated 5′ sequence.

The binding-site for Kid-1 is unknown. In order to establish whether Kid-1 is indeed a transcription factor, fusion proteins between the DNA-binding domain of the yeast transcription factor GAL4 and the non–zinc-finger portion of Kid-1 were employed in transient transfection experiments. The fusion protein thus has the DNA-binding properties of GAL4 and the *trans*-acting properties of Kid-1. Reporter plasmids in these experiments contained either five GAL4 binding-sites, a TATA-box, and a chloramphenicol acetyltransferase (CAT) gene (basal reporter plasmid) or five GAL4 binding-sites, the SV 40 enhancer, a TATA-box, and a CAT gene (SV 40 driven reporter plasmid). In the case of the basal reporter plasmid, the CAT gene is driven only by basal transcription factors. By contrast, in the SV 40–driven reporter plasmid, the CAT gene is under the control of a strong constitutive enhancer, which promotes the assembly of the basal transcription factors over the TATA box and thus enhances transcription of the CAT gene. In either case, cotransfection of a plasmid coding for a GAL4/Kid-1 fusion protein with CAT constructs leads to lower CAT activity, indicating that the non–zinc-finger region of Kid-1 functions as a transcriptional repressor (5) (Figure 7). Genes containing a Kid-1 binding site should therefore be repressed by Kid-1.

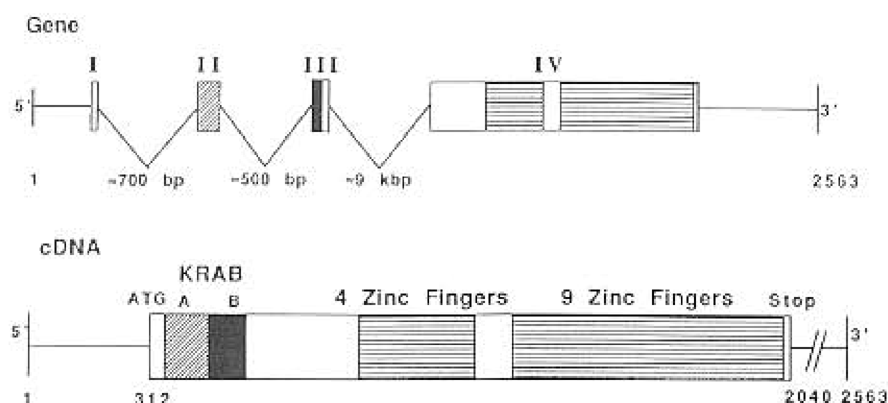

Figure 6 Comparison of the *Kid-1* mRNA and gene. Four exons in the *Kid-1* gene represent the (functional) building blocks of the protein. The main structural motifs in the Kid-1 protein are the Krüppel-associated boxes (KRAB) A and B and the two zinc-finger domains of four and nine zinc fingers each. Exon II encodes the complete KRAB-A domain. Most of exon III consists of KRAB-B encoding sequence. All 13 zinc fingers are encoded by exon IV. (From Ref. 88.)

We have mapped the transcriptional repressor activity of Kid-1 to the KRAB-A domain (89) (Figure 7). KRAB-A and B domains are present in approximately one-third of all zinc-finger proteins and consist of a highly conserved region of approximately 75 amino acids at the NH_2 terminus of these proteins (90). The evolutionary conservation, wide distribution, and genomic organization of the KRAB domain is consistent with an important role of this region in the transcriptional regulatory function of zinc-finger proteins. The KRAB-A domain of another zinc-finger protein, ZNF2, also has repressor activity. Site-directed mutagenesis of conserved amino acids in this motif results in decreased repressor activity. These findings provide a functional significance for the KRAB-A domain.

CONCLUSIONS

The development of the mammalian kidney and its repair after injury are complicated processes resulting from complex genetic programs which are poorly understood. The repair process recapitulates many aspects of the normal developmental process, and hence a better understanding of renal development will likely lead to new experimental paradigms to hasten and modulate the repair process. Developmental processes involve the transformation of a mesenchymal structure into an epithelium with highly specialized functions. Probably many genes have to be turned on or off in that process. We know only a small amount about a minority of these genes, and so far we have little insight into the regulation of these genes during the organogenesis of the kidney. In recent years, a number of transcription factors have been found to be regulated during renal development. The c-*myc* gene is present in all proliferating cells and may have nothing to do with the formation of the kidney per se. N-*myc*, *Pax2*, *Pax-8*, *LFB3*, and *WT-1* are expressed only after an induction process has occurred—that is, after the ureter has invaded the metanephrogenic mesenchyme, has branched, and has caused the condensation of the mesenchymal cells. It is in that condensed mesenchyme that N-*myc*, *Pax2*, *Pax8*, *LFB3*, and *WT-1* appear for the first time. *WT-1* can be assumed to play a necessary though probably not a sufficient role in this earliest morphological transformation. In *WT-1* "knockout mice," kidney development is arrested extremely early. The kidney does not even reach the stage of the condensed mesenchyme. In the case of N-*myc* knockout mice, the situation is less clear, because those animals die prematurely at day 11.5 in utero—that is, before the time of metanephric development. It could be demonstrated, however, that mesonephric development was delayed and disorganized (64). A rather different effect has been noticed in *bcl-2* knockout mice. Bcl-2 is a protein that can prevent apoptosis in many different circumstances. Mice in which both *bcl-2* alleles have been inactivated develop renal failure from polycystic kidney disease (91).

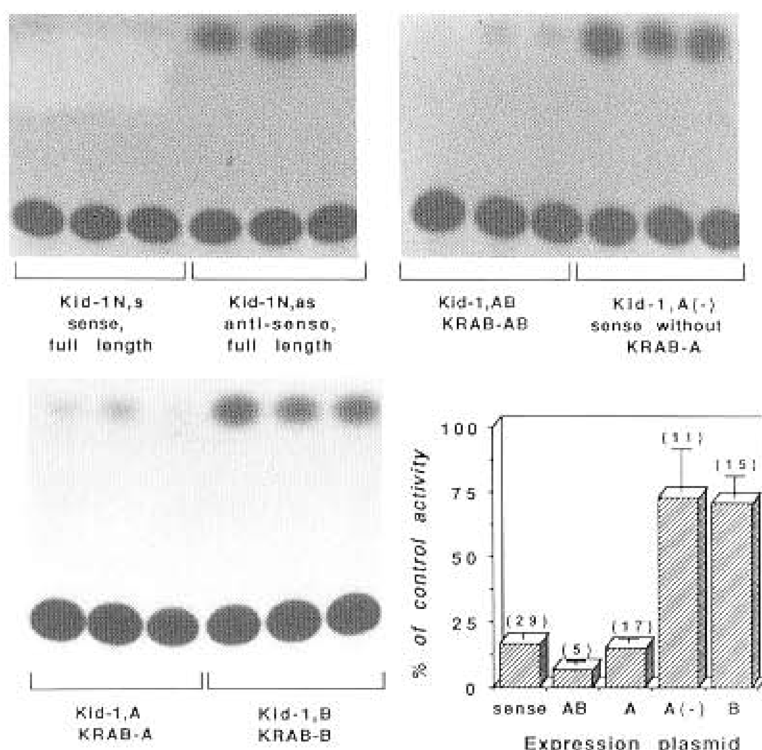

Figure 7 Kid-1 represses transcription—the KRAB-A domain as a transcriptional repressor motif. In transient transfection experiments, the NH_2-terminal, non–zinc-finger region of Kid-1 (Kid-1N,s) strongly represses transcription of a chloramphenicol acetyltransferase (CAT) gene driven by the SV40 promoter. High CAT activity results in the formation of acetylated chloramphenicol, which appears as a second, upper spot on chromatography plates. Deletion mutagenesis identifies the KRAB-A domain as the motif responsible for this repressor effect and defines a novel class of transcriptional repressors.

Expression of fusion proteins containing Kid-1N,s, Kid-1,AB, or Kid-1,A, each of which includes the KRAB-A domain, results in marked repression of CAT activity, when compared to the CAT activity observed in cells transfected with the control antisense construct (Kid-1N,as). In contrast, when the KRAB-A domain is missing from the NH_2-terminus of Kid-1 (Kid-1,A(–) or Kid-1,B), CAT-activity is similar to that of the control antisense plasmid. The graph in the lower right hand corner provides quantitative CAT activity with activity obtained with the antisense Kid-1N,as plasmid taken as 100%. The numbers above the standard error bars indicate the number of independent experiments. (From Ref. 89.)

The time of peak expression is different for each transcription factor, and this timing may provide insights into their regulatory functions. The majority of the discussed transcription factors are undetectable in the fully differentiated kidney, and it can be assumed that they only regulate genes transiently during renal development. This, however, does not necessarily mean that those target genes are not active any longer in the kidneys of the adult animal. *LFB1* and *LFB3* are still present throughout the fully developed tubules. *LFB3* and *Pax2* are expressed in the adult collecting duct system. *WT-1* is expressed in the adult kidney in the podocytes of Bowman's capsule. *Kid-1* is not expressed early in postnatal development and is maximally expressed in adult kidneys.

Though in recent years much has been learned about the genetic control of kidney development, many questions remain unanswered. Which transcription factors are necessary and/or sufficient for the kidney to develop into a functional organ? What are the target genes? What is the exact relationship between renal injury and differentiation? Do any of those transcription factors participate in the pathogenesis of renal cell carcinomas and polycystic kidney disease? Modern molecular and cell

biology techniques such as knockout and transgenic animals, positional cloning, and others will hopefully shed some light on these issues and provide answers to the questions posed.

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