PAX genes in development and disease: the role of PAX2 in urogenital tract development

MICHAEL R. ECCLES*,1, SHUJIE HE, MICHAEL LEGGE1, RAJIV KUMAR1, JODY FOX1, CHAOMING ZHOU, MICHELLE FRENCH and ROBERT W.S. TSAI1

Developmental Genetics Laboratory, Department of Pathology and 1Department of Biochemistry, University of Otago, Dunedin, New Zealand

ABSTRACT PAX genes play an important role in fetal development. Moreover, heterozygous mutations in several PAX genes cause human disease. Here we review the role of PAX2 in kidney development, focusing on the morphological effects of PAX2 mutations. We discuss the role of PAX2 in the context of an inhibitory field model of kidney branching morphogenesis and summarize recent progress in this area.

KEY WORDS: PAX genes, kidney development, Renal-Coloboma Syndrome

Introduction

During development many thousands of genes are expressed to control patterning of the developing embryo. In the early phase of development, very rapid cell proliferation and differentiation occurs, but this rapid growth is under the control of a host of developmental genes. Relatively little is yet known about the processes that control patterning and how the precisely regulated number of cells required to form the complete organism is determined. In this review we describe several experimental approaches designed to improve our understanding of these fundamental problems. This review is based on the work of the Developmental Genetics Laboratory, University of Otago, which is focused on the role of PAX genes in development and cancer. The reason we have chosen to study the PAX family of genes is because individual members of the family control patterning of specific organs, and regulate the decision-making process of cell life and death during development. By understanding the molecular mechanisms of these fundamental processes we hope to gain a better understanding of development and disease, including congenital developmental abnormalities and neoplasia.

PAX Genes

Several gene families have roles in regulating developmental programmes. One such family, called PAX genes (Dahl et al., 1997), derives its name from a conserved DNA sequence motif called the paired box, a conserved 128 amino acid domain in the amino-terminal portion of the protein (Treisman et al., 1991). The PAX genes are a relatively small family of developmental genes that are grouped into four classes on the basis of their structural similarity, depending on sequence homology, the presence or absence of an octapeptide domain, and either a homeodomain or partial homeodomain (Fig. 1) (Dahl et al., 1997). The paired box and homeodomains encode DNA binding domains within the PAX proteins, so each protein is able to act as a transcription factor regulating the expression of a range of downstream genes (Mansouri et al., 1996). An additional domain in the PAX genes is the transactivation domain within the carboxyl terminus of each PAX protein, which is a serine- and threonine-rich domain responsible for transcriptional activation of target genes (Fig. 1) (Ward et al., 1994).

PAX genes have been identified in a wide variety of species, including jellyfish (Sun 1997), the worm C. elegans (Chamberlin et al., 1997), insects such as Drosophila (Quiring et al., 1994), zebrafish (Krauss et al., 1991), chickens (Nohno et al., 1993), and several species of mammal (Noll 1993). Nine PAX genes, PAX1-PAX9, have been described in humans and mice (Dahl et al., 1997). The expression of PAX genes in many tissues during embryogenesis is associated with critical roles for these genes during development (Dahl et al., 1997). In particular, mutations in four of the PAX genes, PAX2, PAX3, PAX6 and PAX8, have been shown to cause human developmental disorders (Table 1) (Dahl et al., 1997).

In mice, null mutations are associated with developmental abnormalities in all nine PAX genes (Table 1). Indeed, mutations in PAX genes are often associated with developmental defects in analogous organ systems in disparate species. For example, Gehring and colleagues showed that a Drosophila PAX gene, "C. elegans, Caenorhabditis elegans, CNS, central nervous system; GFP, green fluorescent protein; 2-D, two dimensional; Pax, paired box; Wt1, Wilms tumor gene 1."
termed eyeless, which is equivalent to PAX6 in mice and humans, is involved in eye development (Quiring et al., 1994). Moreover, the same group in 1995 showed eyeless functions as a master regulatory control gene in Drosophila. When eyeless was artificially expressed in Drosophila out of the context of the normal eye anlagen, such as in wing or leg tissues, it was able to induce the formation of compound eyes on those structures (Gehring and Ikeo 1999, Halder et al., 1995). These observations suggest that fundamentally important functions for PAX genes may have been conserved during evolution.

As mentioned above, the PAX genes have been divided into several subgroups based on sequence homology, and the absence or presence of various sub-domains in the proteins (Fig. 1). The PAX2, PAX5 and PAX8 genes are organized together in group II, as referred to by (Dahl et al., 1997), because their primary sequence is very similar, and each encodes a paired domain, an octapeptide and a partial homeodomain (Ward et al., 1994). A number of PAX genes are also expressed during adulthood, although the distribution of expression is different from that in embryogenesis. For example PAX5 is expressed during haematopoiesis in adult tissues (Adams et al., 1992). PAX genes are also expressed in the adult lens tissue of the eye (Zhang et al., 2001), thymus (Wallin et al., 1996), thyroid (Mansouri et al., 1998), pancreas (Ritz-Laser et al., 2000, Sosa-Pineda et al., 1997, St-Onge et al., 1997), oviduct (Fickenscher et al., 1993), vas deferens, epididymis (Fickenscher et al., 1993, Oefelein et al., 1996), and in myogenic precursors of muscle tissue (Tsukamoto et al., 1994).

**PAX2 in Development and Disease**

The PAX2 gene is expressed during eye, ear, central nervous system (CNS) and urogenital tract development (Dressler et al., 1990, Eccles et al., 1992, Nornes et al., 1990). A critical role for PAX2 in eye, ear, mid/hindbrain and kidney development has been established through the analysis of PAX2 knockout mice (Torres et al., 1995, Torres et al., 1996). Expression of Pax2 in the Wolffian and Mullerian ducts, as well as the branching ureteric bud and induced nephrogenic mesenchyme is consistent with the observation that Pax2 plays a role at an early stage in the patterning of the metanephros (Lechner and Dressler 1997). Pax2 is also expressed in the mesonephros, an earlier transient kidney that is functional for a short time in the fetus. In the developing metanephros, the expression of Pax2 and the related Pax gene, Pax8,
The chromosomal location of \( PAX2 \) in humans did not suggest the possible involvement of \( PAX2 \) in a mapped human genetic disease (Eccles et al., 1992). To identify a syndrome caused by \( PAX2 \) mutations we searched for a disease in humans that would look similar to the disease in \( Krd \) mice (Keller et al., 1994). We identified a \( PAX2 \) mutation in a father and three sons, whose anomalies included optic nerve defects (called optic nerve colobomas), renal anomalies, and vesicoureteric reflux (Fig. 3) (Sanyanusin et al., 1995b). This syndrome is renal-coloboma syndrome, also called papillo-renal syndrome, which is a poorly characterised, recently identified syndrome (Eccles and Schimmenti 1999, Parsa et al., 2001), which has only been reported in 10 families to date. Thirty patients with mutations in various parts of the \( PAX2 \) gene have been identified (Fig. 3). Some of these patients have, in addition to eye and kidney anomalies, high frequency hearing loss and CNS defects (Amiel et al., 2000, Cunliffe et al., 1998, Dureau et al., 2001, Ford et al., 2001, Narahara et al., 1997, Porteous et al., 2000, Sanyanusin et al., 1995a, Schimmenti et al., 1997, Schimmenti et al., 1999). The incidence of renal-coloboma syndrome in the population is unknown but includes as part of the spectrum, primary renal hypoplasia, which is a relatively frequent congenital abnormality. Primary renal hypoplasia, a sub-type of renal hypoplasia, called oligomeganephronia, and renal-coloboma syndrome, have been shown to be caused by \( PAX2 \) mutations (Nishimoto et al., 2001, Salomon et al., 2001). These conditions are associated with potentially life-threatening illness due to the possibility of progression to end-stage renal failure.

**Mouse \( PAX2 \) Mutants**

There are many similarities between renal-coloboma syndrome in humans and the phenotype in heterozygous \( Pax2 \) mutant mice. In humans a frequent mutational site was identified in \( PAX2 \) (Eccles 1998, Eccles and Schimmenti 1999, Sanyanusin et al., 1995a). An identical mutation has been identified in the mouse \( PAX2 \) gene (Favor et al., 1996). In heterozygous mutant animals the disease is very similar to renal-coloboma syndrome in humans, and so may be an exact animal model in which to study the effects of the \( PAX2 \) mutation in urinary tract development. \( Pax2 \) null mutant mice lack any functional \( Pax2 \) protein, and die perinatally, lacking kidneys, ureters, oviducts, vas deferens, and epididymis, and have anomalies of the coxal area as well as defects of the optic nerves and CNS (Favor et al., 1996, Torres et al., 1995, Torres et al., 1996).

Fig. 2. Genomic organization of the human \( PAX2 \) gene. The 12 exons are shown, spanning 86 Kb. The CDNA is shown below the line; the alternatively spliced exon 6 and exon 10 are shown as light blue boxes and the octapeptide domain is shown as a black box.

Fig. 3. Mutations in the human \( PAX2 \) gene. Shown are eight mutations in the various exons of \( PAX2 \). The exons are the same as in Fig. 2. Reproduced with permission from Fig. 2 in Clin. Genet. 56: 1-9 (Eccles and Schimmenti, 1999).
Major Questions in the Field

We are presently interested in several questions about PAX genes and their role in development and disease:
- What is the molecular basis of haploinsufficiency in PAX mutants?
- How does increased or decreased (mutated) PAX gene expression cause disease, and what is the function of PAX proteins?
- What pathways and genes do PAX proteins regulate?
- How do PAX proteins fit into the transcriptional control networks in cells?

The following sections outline several experimental approaches that we are using to address the above questions.

What is the Molecular Basis of Haploinsufficiency?

In humans, heterozygous mutations in PAX2, PAX3 and PAX6 cause developmental abnormalities associated with specific syndromes (Dahl et al., 1997). Heterozygous PAX2 mutations cause renal-coloboma syndrome, as mentioned above. Heterozygous PAX3 mutations in humans cause Waardenburg syndrome, an autosomal dominant syndrome characterized by pigmentary disturbances and sensorineural hearing loss (Tassabehji et al., 1992). Heterozygous mutations in the PAX6 gene cause aniridia, a condition characterised by severe hypoplasia of the iris, accompanied by cataracts and hypoplasia of the ciliary body and retina (Glaser et al., 1992, Jordan et al., 1992). Homologous genetic loci in mice are also associated with naturally occurring PAX gene mutations Pax22Neu (Pax2) (Favor et al., 1996), Splotch (Pax3) (Epstein et al., 1991), and Small eye (Pax6) mutations (Hill et al., 1991). These mice have characteristic abnormalities of the urogenital tract, CNS, coat colouration, nasal structures, eyes, or ears. In mice, as in humans, heterozygous mutations in Pax2, Pax3 and Pax6 cause developmental abnormalities. Little is known about dosage sensitivity with regard to PAX genes.

Dosage sensitivity associated with the PAX genes is of clinical relevance, as syndromes caused by Pax2, Pax3 and Pax6 mutations are inherited in an autosomal dominant fashion (Glaser et al., 1992, Jordan et al., 1992, Sanyanusin et al., 1995b, Tassabehji et al., 1992). However, these syndromes are not truly dominant, and it is better to think of them as semi-dominant. This is because homozygous mutant individuals are more severely affected (often embryonic lethal), than heterozygous mutant individuals (Epstein et al., 1991, Favor et al., 1996, Hill et al., 1991). Phenotypically, this phenomenon is referred to as haploinsufficiency. As with mice, homozygous mutations in Pax3 or Pax6 in humans cause more severe phenotypes. Marriages between people who each carry a heterozygous mutation for the same PAX gene may result in severely affected offspring (Glaser et al., 1994, Zlotogora et al., 1995).

Haploinsufficiency is consistent with the notion that the level of PAX protein required for the full development of certain tissues is important. Another explanation is that there is stochastic allelic inactivation of PAX gene expression in certain tissues. This has been observed for the Pax5 gene, but not for Pax2 (Nutt et al., 1999, Porteous et al., 2000).

Analysis of PAX2 Protein in Kidney and Oviduct Tissues in Pax2 Mutant Mice

The expression of PAX2 protein is detectable by immunohistochemistry in specific cells of the neural tube. Later PAX2 is expressed in the spinal cord, mid/hindbrain, ear, eye, and urogenital system, which includes the Wolffian duct, ureteric bud, collecting duct epithelia, early nephrons, uterus, oviducts, vas deferens, and epididymis. Our recent studies indicate a strong relationship between kidney development and PAX2 expression (Porteous et al., 2000). Therefore, we are interested to determine whether gene dosage, in normal and Pax2 mutant individuals, is mirrored by changes in the level of PAX2 protein expression. Relatively little is known about the PAX2 protein. For instance, is the PAX2 protein post-translationally modified by phosphorylation, and is it of equivalent size and sequence in different tissues where PAX2 is expressed? In human and mouse fetal kidney the PAX2 protein is 48-50 kDa in size (Dressler and Douglass 1992), but it is not known if PAX2 protein is of a similar size in other tissues.

It is not known whether the level of PAX2 protein is precisely regulated in some tissues and not in others? Recent studies suggest that a decreased PAX2 protein level as a result of PAX2 mutation could cause loss or paucity in ureteric bud growth with an excessive amount of apoptosis, especially during a critical window in kidney development (Ostrom et al., 2000, Porteous et al., 2000, Torban et al., 2000). Clinically, renal-coloboma syndrome in humans is associated with small kidneys containing fewer nephrons, and predisposition to renal failure. Heterozygous Pax22Neu mutant mice show a similar phenotypic spectrum to humans with renal-coloboma syndrome, including defects in kidney and eye tissue. Interestingly, abnormal development occurs in some tissues (the ureteric buds and collecting ducts, optic nerve, and cochlea) in heterozygous Pax2 mutants, whereas in other tissues (uterus, oviduct, vas deferens and epididymis) developmental abnormalities occur only in the presence of a homozygous Pax2 mutation. In heterozygous mutant mice the uterus, oviduct, vas deferens and epididymis are unaffected (Table 1).

One possibility is that the PAX2 protein plays different roles during development in different tissues (Table 1). During embryogenesis the kidney develops from the mutual inductive interaction between the metanephric blastema and the ureteric bud, which gives rise to the nephrons, stroma, and collecting system of the kidney. The Wolffian and Mullerian ducts precede development of these structures, and also precede development of the male and female genital tracts. These two ducts are adjacent to each other during embryogenesis, and exhibit strong PAX2 protein expression. We hypothesize that differences in the sensitivity of the oviduct to PAX2 dosage, as compared to kidney tissue, may be due to differences in the PAX2 protein expression in the tissues. Such differences in protein expression may include alternative splicing, post-translational modifications, or differences in sensitivity of the tissue to PAX2 protein level.

To explore whether PAX2 protein is differently expressed in the kidney as compared to oviduct, we have analyzed the protein complement from these tissues by 2-D polyacrylamide protein gel electrophoresis (PAGE). This method resolves more than 1000 proteins and is sensitive to both single changes in single amino acids in proteins, as well as the more subtle changes in charge caused by changes in post-translational modification by glycosylation and phosphorylation. In oviduct and kidney tissues from adult wild type and Pax2 mutant mice (Krd and Pax22Neu mutants), the protein maps from 2-D gels were grossly equivalent. To further resolve Pax2 protein expression using a proteomics approach we are currently using enhanced direct protein staining techniques such as silver staining as well as immunolocalisation.
of Pax2 protein with anti-Pax2 antibody. Ultimately we will undertake comparative analysis of Pax2 protein expression using micro-sequencing techniques and mass spectrometry.

How do Pax Gene Mutations cause their Respective Syndromes?

As discussed above, mutations in several members of the PAX gene family result in developmental abnormalities. In contrast, mutations in highly homologous PAX genes, expressed in the same cell types, do not necessarily cause developmental abnormalities. For example Pax3 and Pax7, which are highly homologous, are co-expressed in developing somites, myogenic precursor cells, and neural crest-derived cells. Heterozygous Pax3 mutations affect neural crest cell migration, and homozygous mutations affect development of the somites, yet Pax7 mutations do not (Borycki et al., 1999, Epstein et al., 1991). What is the basis of this difference?

Another relevant example is that of PAX2 and PAX8, which encode almost identical proteins (Ward et al., 1994). These proteins are co-expressed in several of the same cell types in the developing kidney (Eccles et al., 1995). PAX2 is required for kidney development, while PAX8 is not (Mansouri et al., 1998). Why does one gene result in disease when it is mutated and not the other? There is also a difference between the Pax2 and Pax8 genes in their dosage sensitivity, as Pax8 is expressed in the developing thyroid gland, and homozygous, but not heterozygous, mutations in murine Pax8 cause thyroid gland abnormalities (Mansouri et al., 1998). Kidneys are normal in homozygous Pax8 mutants, yet heterozygous Pax2 mutations cause kidney abnormalities.

Exactly how PAX2 is involved in development of the urogenital tract is unknown. What causes the kidneys of patients with Pax2 mutations to be smaller than normal, and why are these patients predisposed to renal failure? Recently we found that apoptosis in the collecting ducts of Pax2 mutants is greatly increased (Porteous et al., 2000, Torban et al., 2000). However, the precise events orchestrated by Pax2 during fetal growth of the kidneys are not well characterised.

The Role of PAX2 in Ureteric Bud Branching and Development of the Renal Collecting Duct System

Analysis of Kidney Development in Pax2 Mutant GFP Transgenic Mice. Since the discovery of the cause-effect relationship between mutations in Pax2 and renal-coloboma syndrome, we have pursued research to further characterize the functional role of Pax2 and to understand this developmental syndrome. To date, the majority of this research has been in the form of retrospective studies and the data that has been generated has mostly been 2-dimensional, while the process of development itself is 3-dimensional.

Our current research aims to better understand the role of Pax2 in the development of the collecting duct system. We are breeding and crossing several strains of mice, including Pax2<sup>1Neu</sup> mice and Hoxb7/GFP transgenic mice to study branching morphogenesis in the collecting duct system. The latter transgenic mice have been shown to express green fluorescent protein (GFP) during development under the control of the Hoxb7 promoter (Srinivas et al., 1999). Of significance is the GFP expression in the urogenital system, where GFP has been localized in the epithelia of the Wolffian duct, the ureteric bud, and the collecting ducts. This fluorescence can be captured using confocal microscopy, following excitation of the chromophore with a 488 nm laser. This technique allows serial sections to be taken through the fluorescent tissue, and reconstructed into a 3-dimensional image.

We have characterized the development of the metanephric kidney in wildtype and Pax2 mutant mice at different stages of embryological development: E12, E14, E16 and E18. Between E14 and E18 we are using kidney explant organ culture to compare metanephric development between littermates. Using confocal microscopy, it is possible to take time-lapsed images of these cultures, and to analyze metanephric development in wildtype kidneys compared to Pax2 mutant kidneys (Fig. 4). The key question that we are addressing by this procedure is how Pax2<sup>1Neu</sup> mutant fetal mice and wild type littermates differ in the induction of the metanephric mesenchyme by the ureteric bud. A related question is how do these mice differ in the relative rates of proliferation of cells in the collecting ducts, and of apoptosis during development?

Analysis of Kidney Development in Pax2 Mutant Aggregation Chimaera Mice. To investigate the role of Pax2 in kidney development we have generated aggregation chimeras using Pax2<sup>1Neu</sup> and Krd compound heterozygous mutant embryos. The Krd mouse, has a chromosomal deletion of the Pax2 gene and 400cM of flanking DNA (Keller et al., 1994). Unlike Pax2<sup>1Neu</sup> mice, homozygous Krd mutants are preimplantation lethal (Keller et al., 1994). However, heterozygotes of both strains of mice provide good animal models of renal-coloboma syndrome.

The double-mutant embryos are fused to wild type embryos at the 8-cell stage (Fig. 5A). The fused embryos are then cultured to
blastoscyts (see Fig. 5B) before being transferred to pseudopregnant hosts. The chimeras formed by the fusion of the two embryos therefore represent an aggregation of cells of two different genotypes: Pax2<sup>1Neu</sup>/Krd double mutants and wildtype (Fig. 5C). The embryos are then harvested at various times after implantation and the contribution of wild type and mutant cells in kidney development is analyzed.

The generation of aggregation (fusion) chimaera Pax2 mutant mice in this way allows us to determine the mode by which the kidneys fail to develop in homozygous Pax2 mutants. Although Pax2 is clearly a critically required developmental gene, the role of Pax2 in initiating kidney development is difficult to determine because of failed organogenesis and perinatal lethality. Aggregation chimaera mice have mosaicism in all tissues. The percentage of mosaicism for Pax2 double-mutant cells and wild-type normal cells varies from mouse to mouse, but is relatively constant throughout different tissues.

By making aggregation chimaeras we can determine whether Pax2 specifies an early survival function that is required for subsequent kidney development, or whether the missing signal in homozygous Pax2 mutants. Although Pax2 is required for the autonomous survival of kidney epithelial cells, then Pax2 mutant cells would make up a very low percentage of the total complement of cells in the chimaeric kidneys because the mutant cells would die. The kidney would of course be mosaic for mutant and wild-type cells in the chimaeric mice, but there would be a much lower percentage of mutant cells in the kidneys than in the other tissues of the fetus which do not depend on Pax2 expression for cell survival. On the other hand, if Pax2 induces a signal(s) during kidney development, and if wild type cells can complement this signal in the chimaeras, then Pax2 mutant and wild type cells would together make up an equal percentage of cells. Wild type and mutant cells would then contribute equally to the mosaicism of the chimaeric fetal kidneys, and the percentage mosaicism in the kidney would then not be significantly different than in the other tissues of the chimaeric fetal mice.

Conceivably the kidney phenotype of the homozygous mutants might be completely rescued by the presence of wild type cells, and that the percentage of normal cells in the kidneys of the chimaeras would be enough to support normal growth. In addition, we might find that there is a critical time period for the expression of Pax2, after which kidney development is not able to proceed normally.

**How are PAX Genes involved in Cell Survival and Renal Failure?**

Is Pax2 required for kidney cell survival, or is apoptosis simply a default mechanism to eliminate cells that develop abnormally as a result of the PAX2 mutation? We have addressed this question using mIMCD-3 cells. These cells are murine adult kidney collecting duct cells that constitutively express high levels of Pax2. Inhibition of Pax2 expression in mIMCD-3 cells using an antisense PAX2 expression construct causes reduced cell survival. Conversely, enhanced expression of Pax2 in HEK293 human kidney epithelial cells, that do not express Pax2, leads to enhanced cell survival (Torban et al., 2000). We interpret these data to mean that Pax2 is involved in cell survival in mIMCD-3 cells. The mechanism of survival conferred by Pax2 is unknown, but may involve gene regulation.

**Identification of PAX2 Modulated Genes using Microarray and Real Time PCR**

In previous studies we showed that three promoter-luciferase expression constructs, N-myc, p53, and Wilms tumour 1 (WT1) were regulated by Pax2 in transient co-transfection assays (McConnell et al., 1997). We also identified important regions of the WT1 promoter that were regulated by the Pax2 protein. In addition, others have shown that the p53 and WT1 promoters are regulated by Pax2 (Stuart et al., 1995; Dehbi et al., 1996).

To confirm the above findings, and to identify further downstream targets of Pax2, we have undertaken cDNA micro-array experiments using several different experimental systems. Our approach has been to hybridize human or mouse cDNA micro-arrays, containing 6,347 cDNAs and ESTs (purchased from Affymetrix) with probes generated from RNA isolated from human or mouse fetal kidney cells, respectively. HEK293 human fetal kidney cells were stably transfected with a tetracycline regulated PAX2 expression construct. This construct enabled expression of Pax2 in the absence of tetracycline, and reexpression of Pax2 expression when tetracycline was present. HEK293 cells do not normally express Pax2. RNA populations were isolated before and after tetracycline treatment and used to make probes to hybridize to human cDNA microarrays. The results of this experiment are in progress.

In a different microarray experiment we have isolated RNA from embryonic day 16 (E16) Pax2<sup>1Neu</sup>/+ heterozygous mutant and wild type kidneys, and generated probes for microarray hybridization. One cDNA microarray was hybridized with the probe made from the Pax2<sup>1Neu</sup>+/-- mutant fetal kidney RNA, while another cDNA microarray was hybridized with the probe made from wild type fetal kidney RNA. Pax2 gene dosage was halved in these experiments by the heterozygous Pax2 mutation, and therefore not all genes regulated by Pax2 would be likely to be affected. However, because the mutant phenotype shows decreased kidney size and fewer poorly developed nephrons, we expected that at least some
changes in gene expression would be observed, and that some of these changes would be related to the *Pax2*\(^{\Delta N}_{\text{ME}}\) mutant phenotype. To maximize the chances of observing changes in gene expression, kidneys were sorted into small mutant, large mutant, and wild type kidneys by weight and genotype. Kidneys were isolated from fetal mice at E16 and E19.

Genes showing differences of 2-fold or more were identified and the changes in gene expression were validated on the same RNA samples using quantitative Real-Time PCR. The level of expression of each gene was normalized to a simultaneously amplified house-keeping gene, Beta 2 Microglobulin (B2M). Interestingly, several genes in the insulin-like growth factor signaling pathway were changed in expression in the mutant kidneys. There is already some evidence that insulin-like growth factors influence branching morphogenesis and cell survival in the kidney (Feld and Hirschberg 1996, Werner et al., 1994).

**Transcription Factor Networks during Kidney Development: WT1 and Pax2**

The interactions between genes that control growth and differentiation of the embryo, and that may act to limit proliferation by specifying terminal differentiation, are fundamental to understanding the molecular mechanisms of development and oncogenesis. A variety of transcription factors have been identified that are required for development of the kidney (Table 2). However, little is known about the interactions between developmentally regulated transcription factors and the networks of gene regulation that exist to control kidney development. One transcription factor of great interest in urogenital tract development is WT1, encoded by the Wilms tumour suppressor gene locus (Call et al., 1993). There is no expression of WT1 in the developing urinary tract, it is clearly of significance to determine what regulates this gene, and whether these factors may also be important in the development of the urinary tract.

The *Pax2* gene is normally expressed in the mesonephric duct, the ureteric bud, the condensing mesenchyme and in early epithelial structures derived from the mesenchyme, and is quickly down regulated as the tubular epithelium matures (Dehbi et al., 1998). On the other hand, *Pax2* in the mutant mesonephric blastema of WT1-/- mice, but *Pax2* expression is observed in the Wolffian duct and in the mesonephros of WT1-/- mice (Kreidberg et al., 1993). In the epithelial components of human Wilms’ tumors, *PAX2* expression persists (Dressler and Douglass 1992, Eccles et al., 1992), suggesting that negative regulators of *PAX2* are inactive.

Identifying the molecular mechanism of *PAX2* down-regulation during normal nephrogenesis and understanding how these mechanisms are lost during the initiation and progression of renal tumors, as well as how these genes function, may illuminate the genetic basis of renal oncogenesis. It would also be of interest to identify auxiliary proteins that interact with *PAX2* protein and that would therefore have an important role in urinary tract development by being part of a *PAX2* transcription factor complex. WT1

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**Fig. 6.** Graph depicting the expression profiles of the *PAX2* and WT1 genes during development of the basic unit of the kidney, the nephron. The graph shows the level of *PAX2* and WT1 expression as a variable strength band at various stages of kidney development (depicted below the bands). Darker coloring represents stronger expression. Along the horizontal axis are various structures that are observed in fetal kidneys; from left to right; diffuse mesenchyme, condensed mesenchyme, comma-body, S-body, mature nephron. WT1 expression continues in mature nephrons after birth.
duct, and reduced branching morphogenesis.

To explain why apoptosis might reduce branching morphogenesis, and consequently affect the ultimate size of the mature kidney, we have developed an inhibitory field model, which links the control of cell survival to branching morphogenesis (Torban et al., 2000). We postulate that the growing ureteric bud must escape an inhibitory field established by condensing mesenchyme before the next generation of nephrons is produced (Fig. 7). Excessive apoptosis deletes cells from the growing ureteric bud, delaying the escape of the ureteric bud from the field (Fig. 8).

**An Inhibitory Field Model of Kidney Branching Morphogenesis**

As we begin to understand the role of PAX2 and pathways in kidney development, we may learn ways to manipulate the growth of kidneys during embryogenesis, and perhaps even prevent disabling or dysmorphic abnormalities that occur in individuals who carry mutant genes that affect kidney growth and neoplasia. A future focus of the Developmental Genetics Laboratory will be to investigate these possibilities.

**Summary**

**References**


