Published in final edited form as:

Pediatr Nephrol. 2011 September ; 26(9): 1387–1394. doi:10.1007/s00467-010-1749-x.

Patterning and early cell lineage decisions in the developing kidney: the role of Pax genes

Gregory R. Dressler

Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Gregory R. Dressler: dressler@umich.edu

Abstract

Specification of the intermediate mesoderm and the epithelial derivatives that will make the mammalian kidney depends on the concerted action of many transcription factors and signaling proteins. Among the earliest genes expressed in the nephric duct and surrounding mesenchyme is *Pax2*, whose function is essential for making and maintaining the epithelium. The Pax2 protein is subject to phosphorylation in response to signals that activate the c-Jun N-terminal kinase pathway, including Wnts and BMPs. In cell culture systems, Pax2 is known to recruit components of a histone H3 lysine 4 methyltransferase complex to specific DNA sites to alter the pattern of histone modifications and determine gene expression. This epigenetic function may underlie the ability of Pax2 and similar proteins to maintain cell lineages during development.

Keywords

Kidney development; Paired domain; Grg/TLE PTIP; Histone methylation

Introduction

One of the most important papers published in the last 30 years, in my admittedly biased view, was one that I first came across as a graduate student in the early 1980s but did not really appreciate until nearly a decade later. The paper reported research carried out by Nusslein and Wieschaus [1] and was the first to describe a genetic screen in the fruit fly *Drosophila* for mutations that affect body patterning, with an emphasis on segmentation and segment polarity along the anterior–posterior axis. This work has led to the identification of some of the most interesting developmental regulatory gene families and the first conceptualization of the fundamental principles of sequential compartmentalization of a complex developing organism. For their pioneering work, Nusslein and Wieschaus, together with Ed Lewis whose work on homeotic mutations also warranted recognition, were awarded the Nobel Prize in Medicine in 1995.

The work of Nusslein and Wieschaus proved immensely important as many mammalian homologues of *Drosophila* developmental regulatory genes were quickly identified. Genes

such as *hedgehog* and *patch*, *paired*, *hunchback*, *runt*, and *wingless* proved to be the prototypes for families of proteins that controlled not only a whole variety of developmental processes, but were also critical for cancer initiation and progression and other abnormalities. This work was important to me personally, as I began my postdoctoral fellowship in the lab of Peter Gruss, trying to identify the first mouse homologues of the paired gene family. Three of the segmentation genes found in the fly, namely, *paired*, *gooseberry-proximal*, and *gooseberry-distal*, contained a new type of DNA binding domain that was highly conserved across divergent species and allowed us to identify mouse and human genes merely by low-stringency hybridization. Genes encoding this domain were called Pax genes (for paired-box) and were subsequently associated with mammalian development.

In mice and humans, there are nine Pax genes that encode proteins with amino terminal DNA binding, i.e., paired domains. The proteins are classified into four subfamilies according to the presence of additional downstream elements, including an octapeptide repressor domain and a homeobox DNA binding domain (Table 1). All of the Pax genes are expressed in developing structures and control the early specification of specific cell types or the compartmentalization of the embryo into specific regions. Pax genes are among the many types of prototypical DNA binding proteins that evolved exclusively in metazoans and are likely to be important for cell lineage specification in multicellular organisms [2]. For example, *Pax6*, the human *aniridia* gene, was proposed to encode a master regulatory switch for eye development because of its ability to induce ectopic eyes in *Drosophila* [3] and in vertebrates [4]. While this may be an oversimplification, the idea that Pax genes are genetic switches for cell lineage decisions is supported by many lines of evidence. For example, in the lymphoid lineage, *Pax5* determines the B cell phenotype [5], whereas in the kidney *Pax2* appears to determine the epithelial cell phenotype.

Pax genes in kidney development

In the kidney, *Pax2* is essential for initiating the development of the mesonephros and metanephros from the intermediate mesoderm. After gastrulation, the mesoderm is compartmentalized along the medio-lateral axis into axial, intermediate, and lateral plate mesoderm (for more comprehensive reviews of kidney development, see [6, 7]). Among the earliest markers specific for the intermediate mesoderm are the expression of the *Pax2* and *Pax8* genes, which are considered to be redundant at embryonic day 9.5 (E9.5) in the mouse [8]. However, at later stages, *Pax2*—but not *Pax8*—mutant mice exhibit complete agenesis of both kidneys and ureters [9]. In humans, the loss of one *Pax2* allele can result in renal hypoplasia, vesicoureteral reflux, and optic nerve colobomas [10-12]. These phenotypes are consistent with the *Pax2* expression pattern in the epithelial components of the mesonephros, the Wolffian duct, and the ureteric bud and in the developing renal tubules [13, 14]. Both in vivo and in vitro, *Pax2* is required for the conversion of renal mesenchymal cells to a primitive, proliferating epithelium [15]. Prior to induction, Pax2 protein expression demarcates the metanephric mesenchyme, activates glial-derived neurotrophic factor (GDNF) expression such that ureteric bud epithelial outgrowth can occur, and controls the response to inductive signals [16]. In the developing nephrons, *Pax2* expression persists in the condensing cap mesenchyme around the ureteric bud tips and in the comma and s-

shaped bodies, being down-regulated first in the most proximal loop of the s-shaped body and then in the epithelial cells of the proximal and distal tubules (Fig. 1a).

The cumulative genetic and expression data point to an essential role for *Pax2* in patterning the early intermediate mesoderm such that renal epithelial cells can be generated. In the chick embryo, ectopic *Pax2* could expand the region of intermediate mesoderm [8], whereas in the *Xenopus* embryo *Pax2* and *lim1* could achieve similar effects [17]. Thus, within the context of the developing mesoderm, *Pax2* may be sufficient to define the boundaries of the intermediate mesoderm and the kidney progenitor field along the medio-ventral axis.

Pax genes in disease

In addition to their fundamental roles during embryonic development, Pax genes also contribute to the initiation and progression of disease. In cancer cells, *Pax2* is aberrantly expressed in human Wilms' tumors [14], in adult renal cell carcinoma [18, 19], and in prostate carcinomas [20], and it may be a necessary for tumor growth [18]. *Pax2* expression is also linked to endometrial cancer in response to tamoxifen and estrogen [21], consistent with the early expression and function of *Pax2* in the Mullerian ducts [9].

Persistent *Pax2* expression is observed in other diseases characterized by the aberrant proliferation of renal epithelial cells, such as polycystic kidney disease (PKD) [22] and infantile cystic-dysplastic kidneys [23]. In two different mouse models of PKD, reduced *Pax2* gene dosage slows renal cyst growth by increasing the rate of apoptosis in cystic epithelia [22, 24], suggesting that *Pax2* regulates essential survival factors for proliferating epithelia. Unlike the proximal and distal tubules, in adult inner medullary collecting ducts, *Pax2* expression persists and is up-regulated under hyperosmotic conditions to promote survival [25]. In contrast, persistent expression in proximal tubules and glomerular podocytes in transgenic mice results in microcystic kidneys and nephrotic syndrome [26]. While *Pax2* expression in mature non-dividing proximal tubular epithelial cells is undetectable, *Pax2* expression persists in other urogenital epithelia, such as the endometrial glands of the uterus where its loss, together with the tumor suppressor PTEN, is associated with endometrial precancerous lesions [27]. Thus, both the gain or loss of Pax expression can be deleterious in developing and in adult organisms.

The biochemistry of Pax protein function

While the genetics of Pax genes has clearly established their biological roles in morphogenesis, how the proteins achieve their biochemical functions is still unclear. The Pax proteins interact with DNA through the characteristic 128 amino acid paired domain, located at the amino-terminus of the protein that binds a bipartite DNA recognition sequence [28-31]. The crystal structure of the *Drosophila* paired protein bound to DNA [32] shows three amino-terminal α -helices that resemble a homeo-domain, followed by a carboxyl terminal region with three smaller α -helices. The amino terminal α -helices contact the 3' part of the bipartite target sequence, whereas the carboxyl terminal tail recognizes the 5' end of the DNA sequence. Furthermore, interactions between the paired domain and the DNA recognition sequences can change the conformation of the Pax protein and the target DNAs

[33, 34]. The size and complexity of the DNA binding domain found in Pax proteins has made it difficult to discern consensus binding sites, although several have been proposed.

Genes thought to be up regulated by *Pax2* include *Wt1* [35], *gdnf* [16], *Wnt4* [36], and *sfrp2* [37] in the developing kidney and *engrailed-2* [38] in the developing hindbrain. Yet, these may represent only a small fraction of the potential *Pax2* target genes. In cell culture systems, *Pax2*-dependent transcription activation requires the serine–threonine-rich carboxyl-terminal domain [39], which can be phosphorylated by the c-Jun N-terminal kinase (JNK) to enhance transactivation of *Pax2* reporter genes [40]. JNK translocates to the nucleus in response to a variety of external signals, including Wnt proteins, and phosphorylates specific nuclear transcription factors [41]. In the kidney, the most relevant upstream activators of JNK may be *Wnt4* and *Wnt9b*, which are known regulators of kidney development [42, 43]. The activation of JNK has been observed in cultured nephron progenitor cells [44] and also in response to bone morphogenetic proteins (BMPs) in whole embryonic kidney organ cultures [45]. The *Pax2* protein also associates with the JNK scaffolding protein Jip1, and this interaction is enhanced upon activation of the JNK signaling module [40]. Compound mutants of *JNK1*, 2, and 3 show renal hypoplasia and optic nerve coloboma very similar to *Pax2* hypomorphic phenotype [46]. Furthermore, inhibition of JNK activity by pharmacological inhibitors can inhibit renal development in vitro (Fig. 2). JNK inhibition results in *Pax2*-positive metanephric mesenchymal cells that fail to aggregate around the tips of the ureteric buds. Clearly, JNK activation can result in the phosphorylation of many other nuclear effector proteins, such as c-Jun and ATF. Yet these data suggest an attractive model whereby Wnt signaling drives increased phosphorylation of the *Pax2* transactivation domain to stimulate *Pax2*-dependent gene expression in response to inductive signals. However, the identification of specific sites of phosphorylation on the *Pax2* protein and confirmation that such sites are indeed phosphorylated in vivo will be necessary to define the mechanism of JNK function in the developing kidney.

Pax proteins also suppress gene expression by interactions with Groucho (Grg/Tle) proteins [47]. Grg4 suppresses *Pax2*-dependent gene activation in cell culture, can form a complex at the DNA binding site, and can completely suppress phosphorylation of the *Pax2* transactivation domain, even in the presence of activated JNKs [48]. Long-term repression by Groucho is thought to involve interactions with histone deacetylases and the chromatin silencing machinery [49, 50]. However, deacetylation of histones is not the only possible mechanism of Groucho-mediated repression, as experiments with deletion mutants [51], deacetylase inhibitors [49], and anti-HDAC antibodies [52] would suggest. Grg4 expression is found at low levels in the kidney mesenchyme, including *Pax2*-positive cap mesenchyme (Fig. 1b). However, as nephron development progresses, Grg4 is up-regulated in the s-shaped bodies in podocyte progenitor cells and persists in the more mature podocytes.

The Pax proteins and histone modification

Despite the well-characterized genetics and structural biology, few *Pax* proteins have been directly linked to the transcription machinery. Until recently, most assays for *Pax* activity in cells utilized transient reporters outside the context of chromatin. However, it is now

possible to to model Pax2 activity along different lines (Fig. 3). An emerging hypothesis is that Pax proteins mark specific regions of chromatin for histone modification. Such epigenetic marks can delineate both active and inactive regions of the genomes in a stable and heritable manner. Thus, cell lineage restriction becomes fixed at the level of chromatin structure, thereby establishing chromatin in an active or repressed state such that transcription or silencing can be maintained in subsequent generations of daughter cells.

Chromatin biology and the study of epigenetic pathways have progressed due to the remarkable progress made in defining the patterns of histone modifications and identifying the enzymes responsible for the methylation and acetylation of histone tails. While the Polycomb and Trithorax group of genes were defined genetically as repressors and activators, respectively, in flies, the true biochemical function of these proteins was not evident until the purification of histone methyltransferases and their associated proteins in yeast and tetrahymena (for review see [53]). Thus, many of the Polycomb group proteins are part of complexes that methylate histone H3 at lysine 9 or lysine 27, or histone H4 at lysine 20; these modifications correlate with gene silencing and heterochromatin formation. The Trithorax group of proteins generally belong to complexes associated with histone H3 lysine 4 (H3K4) or lysine 36 (H3K36) methylation, modifications usually associated with active gene expression and euchromatin. These specific lysine residues can be mono-, di-, or trimethylated, further increasing the combinatorial complexity of nucleosome modifications.

The modification of histones was first linked to cell lineage decisions in embryonic stem cells by large-scale genomic chromatin immunoprecipitation analyses with antibodies specific for trimethylation at H3K4 and H3K27. In pluripotent embryonic stem (ES) cells, many key regulatory genes had low levels of both types of methylation marks, even though the genes were not expressed [54, 55]. These so-called bivalent epigenetic marks were then resolved into high levels of trimethyl H3K4 or H3K27 depending on whether the genes were expressed or not in subsequent differentiated derivatives. These data are consistent with a model of cell lineage decision-making that requires the compartmentalization of the genome into active and inactive domains. In mammalian development, the loss of pluripotency occurs as the epiblast undergoes gastrulation to form the primary germ layers. Strikingly, many of the epigenetic regulatory genes, including Polycomb and Trithorax homologues and de novo DNA methylases, show embryonic defects at this time.

If an accumulation of positive and negative epigenetic marks is essential for differentiation along cell lineage pathways, then specific proteins must control the locus and tissue specificity for Polycomb and Trithorax complexes. In flies, the Polycomb response elements (PREs) are known *cis*-acting DNA sequences that bind directly to the complex [56, 57]. However, in mammals, PREs have not been described, nor is it clear how histone methyltransferase complexes recognize individual genes at the right time.

The identification of the Pax2 interacting protein PTIP led us to re-examine the biochemical function of Pax proteins in development. PTIP is a ubiquitously expressed nuclear protein that is part of an H3K4 methylation complex and contains a carboxy-terminal phosphoserine binding domain [58-61]. In cell culture, Pax2 binding to DNA recruits PTIP and a KMT2C/D complex that methylates H3K4 at that site [61]. The KMT2s are SET domain

proteins that are the mammalian homologues of the *Drosophila* epigenetic regulator Trithorax. The recruitment of KMT2D requires PTIP, suggesting that the Pax–PTIP interaction is a rate-limiting step in nucleating the complex (Fig. 3). Thus, PTIP acts as an adaptor protein that links the H3K4 methylation machinery to a sequence-specific binding protein. Although there are multiple Trithorax homologues in mammals, PTIP was not found in the KMT2A (MLL1) complex that was first purified, rather it seems to co-purify with the KMT2C/D complexes, suggesting that there may be other adaptor molecules yet to be discovered.

Mice homozygous for a PTIP null allele are post-gastrulation lethal, disorganized, and developmentally arrested [62]. This phenotype is more severe than any of the KMT2 mutations described to date. By E8.5 there is a global reduction in detectable levels of H3K4 di- and trimethylation. These data suggest that PTIP must interact with other DNA binding proteins beyond the Pax family. This is confirmed in the *Xenopus* gastrula where a PTIP homologue was shown to mediate activin signaling by binding to P-Smad2 [63]. In the fly embryo, a PTIP homologue is also essential for early patterning, including the correct expression of segmentation genes prior to gastrulation and the activation of many Polycomb group target genes once repression is relieved [64]. We have also studied PTIP in embryonic stem cells, where it is necessary for retaining pluripotency during in vitro culture [65].

The *Pax2* gene has a close relative, *Pax5*, whose paired domain is identical and whose biochemical function is likely to be very similar because the two proteins can complement each other [66]. Given that *Pax5* specifies B cell fate in the lymphoid lineage, we recently examined the function of PTIP in B cells. Consistent with the previous data linking PTIP to H3K4 methylation, PTIP-deficient B cells are unable to methylate the promoter regions necessary for immunoglobulin class switch recombination [67], a process that requires Pax5 protein and long-range chromatin interactions.

Our hypothesis is that Pax proteins mark specific regions of chromatin through the modification of histones (Fig. 3). These epigenetic marks can delineate both active and inactive regions of the genomes in a stable and heritable manner through potential interactions with nucleosome remodeling complexes and other chromatin effectors [68]. In the case of Pax2, we propose that interactions with PTIP specify active regions of chromatin, whereas interactions with the Grg/Tle family or repressors could delineate silent regions of chromatin. One advantage of such a model is that a given gene could be activated early, when levels of Grg/Tle are low, and silenced as Grg/Tle levels rise. In the developing intermediate mesoderm and the kidney, this could establish chromatin domains in an active state such that embryonic gene transcription can occur in epithelial progenitors and subsequent generations of proliferating cells. Thus, the Pax2-positive cap-mesenchyme, surrounding the ureteric bud tips, could be imprinted with an epithelial fate in response to inductive signals. Since PTIP and the KMT2 proteins are expressed in all cells, the control is achieved by the activation of a DNA binding proteins that targets the histone methylation complex to specific genes. In order to examine this hypothesis, new tools and methods must be developed such that Pax2 target genes can be identified and analyzed at the level of chromatin structure.

Summary

The specification of cell lineages and patterning in the embryo occurs sequentially as specific regions are increasingly restricted in their developmental fates. When and how this occurs is still not entirely clear. Nevertheless, the role of epigenetic regulatory genes in partitioning the genome into active and inactive domains is evident in a variety of organisms and is highly conserved through evolution. The function of Pax2 in the kidney has been inferred by the phenotypic analysis of loss-of-function mutants in mice, fish, and humans. Our recent data suggest that Pax proteins help establish the renal epithelial fate by delineating chromatin domains through histone modification. The novel protein PTIP is a key adaptor that links Pax proteins and possibly many other types of DNA binding proteins to a histone H3K4 methyltransferase complex. If such modifications are established in development, we now need to address the effects of epigenetic changes on renal disease states, on the stability of the terminal epithelial phenotype, and in the aging cell.

Acknowledgments

I thank S. Patel, and the members of my laboratory, H. Xiao, K. Schwab, and A. Soofi for valuable discussions. This work is supported in part by NIH grants DK054740 and DK073722 to G.R.D.

References

1. Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature*. 1980; 287:795–801. [PubMed: 6776413]
2. Degan BM, Vervoort M, Larroux C, Richards GS. Early evolution of metazoan transcription factors. *Curr Opin Genet Dev*. 2009; 19:591–599. [PubMed: 19880309]
3. Halder G, Callaerts P, Gehring WJ. Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science*. 1995; 267:1788–1792. [PubMed: 7892602]
4. Chow RL, Altmann CR, Lang RA, Hemmati-Brivanlou A. Pax6 induces ectopic eyes in a vertebrate. *Development*. 1999; 126:4213–4222. [PubMed: 10477290]
5. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. *Nat Immunol*. 2007; 8:463–470. [PubMed: 17440452]
6. Dressler GR. The cellular basis of kidney development. *Annu Rev Cell Dev Biol*. 2006; 22:509–529. [PubMed: 16822174]
7. Dressler GR. Advances in early kidney specification, development and patterning. *Development*. 2009; 136:3863–7384. [PubMed: 19906853]
8. Bouchard M, Souabni A, Mandler M, Neubuser A, Busslinger M. Nephric lineage specification by Pax2 and Pax8. *Genes Dev*. 2002; 16:2958–2970. [PubMed: 12435636]
9. Torres M, Gomez-Pardo E, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. *Development*. 1995; 121:4057–4065. [PubMed: 8575306]
10. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont MEM, Sullivan MJ, Dobyns WB, Eccles MR. Mutation of the *Pax2* gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nat Genet*. 1995; 9:358–364. [PubMed: 7795640]
11. Sanyanusin P, McNoe LA, Sullivan MJ, Weaver RG, Eccles MR. Mutation of *PAX2* in two siblings with renal-coloboma syndrome. *Hum Mol Genet*. 1995; 4:2183–2184. [PubMed: 8589702]
12. Schimmenti LA, Cunliffe HE, McNoe LA, Ward TA, French MC, Shim HH, Zhang YH, Proesmans W, Leys A, Byerly KA, Braddock SR, Masuno M, Imaizumi K, Devriendt K, Eccles MR. Further delineation of renal-colombo syndrome in patients with extreme variability of phenotype and identical *PAX2* mutations. *Am J Hum Genet*. 1997; 60:968–978.

13. Dressler GR, Deutsch U, Chowdhury K, Nornes HO, Gruss P. *Pax2*, a new murine paired-box containing gene and its expression in the developing excretory system. *Development*. 1990; 109:787–795. [PubMed: 1977574]
14. Dressler GR, Douglass EC. *Pax-2* is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. *Proc Natl Acad Sci USA*. 1992; 89:1179–1183. [PubMed: 1311084]
15. Rothenpieler UW, Dressler GR. *Pax-2* is required for mesenchyme-to-epithelium conversion during kidney development. *Development*. 1993; 119:711–720. [PubMed: 8187639]
16. Brophy PD, Ostrom L, Lang KM, Dressler GR. Regulation of ureteric bud outgrowth by Pax2-dependent activation of the glial derived neurotrophic factor gene. *Development*. 2001; 128:4747–4756. [PubMed: 11731455]
17. Carroll TJ, Vize PD. Synergism between Pax-8 and lim-1 in embryonic kidney development. *Dev Biol*. 1999; 214:46–59. [PubMed: 10491256]
18. Gnarr JR, Dressler GR. Expression of Pax-2 in human renal cell carcinoma and growth inhibition by antisense oligonucleotides. *Cancer Res*. 1995; 55:4092–4098. [PubMed: 7664285]
19. Daniel L, Lechevallier E, Giorgi R, Sichez H, Zattara-Cannoni H, Figarella-Branger D, Coulange C. Pax-2 expression in adult renal tumors. *Hum Pathol*. 2001; 32:282–287. [PubMed: 11274636]
20. Khoubehi B, Kessler AM, Adshead JM, Smith GL, Smith RD, Ogden CW. Expression of the developmental and oncogenic Pax2 gene in human prostate cancer. *J Urol*. 2001; 165:2115–2120. [PubMed: 11371938]
21. Wu H, Chen Y, Liang J, Shi B, Wu G, Zhang Y, Wang D, Li R, Yi X, Zhang H, Sun L, Shang Y. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. *Nature*. 2005; 438:981–987. [PubMed: 16355216]
22. Ostrom L, Tang MJ, Gruss P, Dressler GR. Reduced Pax2 gene dosage increases apoptosis and slows the progression of renal cystic disease. *Dev Biol*. 2000; 219:250–258. [PubMed: 10694420]
23. Winyard PJD, Risdon RA, Sams VR, Dressler GR, Woolf AS. The PAX2 transcription factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. *J Clin Invest*. 1996; 98:451–459. [PubMed: 8755656]
24. Stayner C, Iglesias DM, Goodyer PR, Ellis L, Germino G, Zhou J, Eccles MR. *Pax2* gene dosage influences cystogenesis in autosomal dominant polycystic kidney disease. *Hum Mol Genet*. 2006; 15:3520–3528. [PubMed: 17082250]
25. Cai Q, Dmitrieva NI, Ferraris JD, Brooks HL, van Balkom BW, Burg M. Pax2 expression occurs in renal medullary epithelial cells in vivo and in cell culture, is osmoregulated, and promotes osmotic tolerance. *Proc Natl Acad Sci USA*. 2005; 102:503–508. [PubMed: 15623552]
26. Dressler GR, Wilkinson JE, Rothenpieler UW, Patterson LT, Williams-Simons L, Westphal H. Deregulation of *Pax-2* expression in transgenic mice generates severe kidney abnormalities. *Nature*. 1993; 362:65–67. [PubMed: 8383297]
27. Monte NM, Webster KA, Neuberg D, Dressler GR, Mutter GL. Joint loss of PAX2 and PTEN expression in endometrial precancers and cancer. *Cancer Res*. 2010; 70:6225–6232. [PubMed: 20631067]
28. Czerny T, Schaffner G, Busslinger M. DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. *Genes Dev*. 1993; 7:2048–2061. [PubMed: 8406007]
29. Czerny T, Busslinger M. DNA-binding and transactivation properties of Pax-6: three amino acids in the paired domain are responsible for the different sequence recognition of Pax-6 and BSAP (Pax-5). *Mol Cell Biol*. 1995; 15:2858–2871. [PubMed: 7739566]
30. Epstein JA, Glaser T, Cai J, Jepeal L, Walton DS, Maas RL. Two independent and interactive DNA-binding subdomains of the Pax6 paired domain are regulated by alternative splicing. *Genes Dev*. 1994; 8:2022–2034. [PubMed: 7958875]
31. Phelps DE, Dressler GR. Identification of novel Pax-2 binding sites by chromatin precipitation. *J Biol Chem*. 1996; 271:7978–7985. [PubMed: 8626478]
32. Xu W, Rould MA, Jun S, Desplan C, Pabo CO. Crystal structure of a paired domain-DNA complex at 2.5 Å resolution reveals structural basis for Pax developmental mutations. *Cell*. 1995; 80:639–650. [PubMed: 7867071]

33. Epstein J, Cai J, Glaser T, Jepeal L, Maas R. Identification of a Pax paired domain recognition sequence and evidence for DNA-dependent conformational changes. *J Biol Chem.* 1994; 269:8355–8361. [PubMed: 8132558]
34. Chalepakis G, Wijnholds J, Gruss P. Pax-3-DNA interaction: flexibility in the DNA binding and induction of DNA conformational changes by paired domains. *Nucleic Acids Res.* 1994; 22:3131–3137. [PubMed: 8065927]
35. Dehbi M, Ghahremani M, Lechner M, Dressler G, Pelletier J. The paired-box transcription factor, PAX2, positively modulates expression of the Wilms' tumor suppressor gene (WT1). *Oncogene.* 1996; 13:447–453. [PubMed: 8760285]
36. Torban E, Dziarmaga A, Iglesias D, Chu LL, Vassilieva T, Little M, Eccles M, Discenza M, Pelletier J, Goodyer P. PAX2 activates WNT4 expression during mammalian kidney development. *J Biol Chem.* 2006; 281:12705–12712. [PubMed: 16368682]
37. Brophy PD, Lang KM, Dressler GR. The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor. *J Biol Chem.* 2003; 278:52401–52405. [PubMed: 14561758]
38. Song DL, Chalepakis G, Gruss P, Joyner AL. Two Pax-binding sites are required for early embryonic brain expression of an Engrailed-2 transgene. *Development.* 1996; 122:627–635. [PubMed: 8625814]
39. Lechner MS, Dressler GR. Mapping of Pax-2 transcription activation domains. *J Biol Chem.* 1996; 271:21088–21093. [PubMed: 8702876]
40. Cai Y, Lechner MS, Nihalani D, Prindle MJ, Holzman LB, Dressler GR. Phosphorylation of Pax2 by the c-Jun N-terminal kinase and enhanced Pax2-dependent transcription activation. *J Biol Chem.* 2002; 277:1217–1222. [PubMed: 11700324]
41. Davis RJ. Signal transduction by the JNK group of MAP kinases. *Cell.* 2000; 103:239–252. [PubMed: 11057897]
42. Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature.* 1994; 372:679–683. [PubMed: 7990960]
43. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell.* 2005; 9:283–292. [PubMed: 16054034]
44. Osafune K, Takasato M, Kispert A, Asashima M, Nishinakamura R. Identification of multipotent progenitors in the embryonic mouse kidney by a novel colony-forming assay. *Development.* 2006; 133:151–161. [PubMed: 16319116]
45. Blank U, Brown A, Adams DC, Karolak MJ, Oxburgh L. BMP7 promotes proliferation of nephron progenitor cells via a JNK-dependent mechanism. *Development.* 2009; 136:3557–3566. [PubMed: 19793891]
46. Weston CR, Wong A, Hall JP, Goad ME, Flavell RA, Davis RJ. JNK initiates a cytokine cascade that causes Pax2 expression and closure of the optic fissure. *Genes Dev.* 2003; 17:1271–1280. [PubMed: 12756228]
47. Eberhard D, Jimenez G, Heavey B, Busslinger M. Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. *EMBO J.* 2000; 19:2292–2303. [PubMed: 10811620]
48. Cai Y, Brophy PD, Levitan I, Stifani S, Dressler GR. Groucho suppresses Pax2 transactivation by inhibition of JNK-mediated phosphorylation. *EMBO J.* 2003; 22:5522–5529. [PubMed: 14532124]
49. Courey AJ, Jia S. Transcriptional repression: the long and the short of it. *Genes Dev.* 2001; 15:2786–2796. [PubMed: 11691830]
50. Chen G, Courey AJ. Groucho/TLE family proteins and transcriptional repression. *Gene.* 2000; 249:1–16. [PubMed: 10831834]
51. Fisher AL, Caudy M. Groucho proteins: transcriptional corepressors for specific subsets of DNA-binding transcription factors in vertebrates and invertebrates. *Genes Dev.* 1998; 12:1931–1940. [PubMed: 9649497]

52. Dasen JS, Barbera JP, Herman TS, Connell SO, Olson L, Ju B, Tollkuhn J, Baek SH, Rose DW, Rosenfeld MG. Temporal regulation of a paired-like homeodomain repressor/TLE corepressor complex and a related activator is required for pituitary organogenesis. *Genes Dev.* 2001; 15:3193–3207. [PubMed: 11731482]
53. Kouzarides T. Chromatin modifications and their function. *Cell.* 2007; 128:693–705. [PubMed: 17320507]
54. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell.* 2006; 125:315–326. [PubMed: 16630819]
55. Azuara V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM, Gouti M, Casanova M, Warnes G, Merkenschlager M, Fisher AG. Chromatin signatures of pluripotent cell lines. *Nat Cell Biol.* 2006; 8:532–538. [PubMed: 16570078]
56. Ringrose L, Paro R. Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development.* 2007; 134:223–232. [PubMed: 17185323]
57. Schwartz YB, Pirrotta V. Polycomb complexes and epigenetic states. *Curr Opin Cell Biol.* 2008; 20:266–273. [PubMed: 18439810]
58. Manke IA, Lowery DM, Nguyen A, Yaffe MB. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science.* 2003; 302:636–639. [PubMed: 14576432]
59. Issaeva I, Zonis Y, Rozovskaia T, Orlovsky K, Croce CM, Nakamura T, Mazo A, Eisenbach L, Canaani E. Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. *Mol Cell Biol.* 2007; 27:1889–1903. [PubMed: 17178841]
60. Cho YW, Hong T, Hong S, Guo H, Yu H, Kim D, Guszczynski T, Dressler GR, Copeland TD, Kalkum M, Ge K. PTIP associates with MLL3- and MLL4-containing histone H3 lysine 4 methyltransferase complex. *J Biol Chem.* 2007; 282:20394–20406.
61. Patel SR, Kim D, Levitan I, Dressler GR. The BRCT-domain containing protein PTIP links PAX2 to a histone H3, lysine 4 methyltransferase complex. *Dev Cell.* 2007; 13:580–592. [PubMed: 17925232]
62. Cho EA, Prindle MJ, Dressler GR. BRCT domain-containing protein PTIP is essential for progression through mitosis. *Mol Cell Biol.* 2003; 23:1666–17163. [PubMed: 12588986]
63. Shimizu K, Bourillot PY, Nielsen SJ, Zorn AM, Gurdon JB. Swift is a novel BRCT domain coactivator of Smad2 in transforming growth factor beta signaling. *Mol Cell Biol.* 2001; 21:3901–3912. [PubMed: 11359898]
64. Fang M, Ren H, Liu J, Cadigan KM, Patel SR, Dressler GR. Drosophila ptip is essential for anterior/posterior patterning in development and interacts with the PcG and trxB pathways. *Development.* 2009; 136:1929–1938. [PubMed: 19429789]
65. Kim D, Patel SR, Xiao H, Dressler GR. The role of PTIP in maintaining embryonic stem cell pluripotency. *Stem Cells.* 2009; 27:1516–1523. [PubMed: 19544464]
66. Bouchard M, Pfeffer P, Busslinger M. Functional equivalence of the transcription factors Pax2 and Pax5 in mouse development. *Development.* 2000; 127:3703–3713. [PubMed: 10934015]
67. Daniel JA, Santos MA, Wang Z, Zang C, Schwab KR, Jankovic M, Filsuf D, Chen HT, Gazumyan A, Yamane A, Cho YW, Sun HW, Ge K, Peng W, Nussenzweig MC, Casellas R, Dressler GR, Zhao K, Nussenzweig A. PTIP promotes chromatin changes critical for immunoglobulin class switch recombination. *Science.* 2010; 329:917–923. [PubMed: 20671152]
68. Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. *Nat Rev Mol Cell Biol.* 2007; 8:983–994. [PubMed: 18037899]

Fig. 1.

Expression of paired-box 2 (Pax2) and groucho-related 4 (Grg4) protein in the embryonic kidney. Immunostaining for proteins in serial sagittal, sections from an embryonic day 16.5 (E16.5) mouse kidney. **a** The Pax2 protein is shown in *red* and E-cadherin is given in *green*. Note high levels of Pax2 protein in the peripheral nephrogenic zone, especially the cap mesenchyme (*arrows*). Note also that Pax2 levels decrease in the s-shaped bodies as the podocyte progenitors are specified (*arrowheads*). **b** The Grg4 protein is shown in *red* and E-cadherin is given in *green*. Note the low levels in nuclei of mesenchymal cells (*arrowheads*) and high levels in podocyte progenitor cells (*arrowheads*)

Fig. 2.

Effect of c-Jun N-terminal kinase (JNK) inhibitors on kidney development in vitro. Kidney rudiments dissected from E11.5 mouse embryos were cultured for 2 days either with control media (**a**) or with 10 μ M of SP600125, a specific JNK inhibitor (**b**). Whole rudiments were stained with antibodies against Pax2 (*red*) or cytokeratin (*green*). Cytokeratin marks the ureteric bud and its branches. Note the condensation of Pax2-positive cells around the ureteric bud tips in **a** (*arrows*). Upon JNK inhibition, Pax2-positive cells are diffuse and fail to aggregate (**b**, *arrows*)

Fig. 3.

Model of Pax2-mediated chromatin remodeling. Pax2 binds to a target DNA sequence and interacts with the adaptor protein PTIP when Pax2 is phosphorylated. PTIP recruits a KMT2 and promotes H3K4 methylation. This enables nucleosome remodeling factors (*NuRFs*) to keep the chromatin open and allow for nucleosome movement. Under conditions of increasing Groucho (*Grg/Tle*) concentration, Pax2 binds to a Grg/Tle complex, which dephosphorylates the Pax2 carboxy-terminal domain and inhibits PTIP interactions. The Grg/Tle complex may then promote silencing by recruiting chromatin condensation proteins such as HP1

Table 1

The Pax (paired-box) gene family

Gene	Chromosome		Structural Features*				Mutations		Expression Patterns
	Mouse	Human	PD	OD	HD		Mouse	Man	
Pax1	2	20p					Undulated		vertebral column, thymus
Pax2	19	10q						Renal Coloboma	kidney, CNS, eye, ear
Pax3	1	2q					Spotch	Wardenburg	CNS, neural crest, nose, muscle
Pax4	6	7
Pax5	4	9p							CNS, B-lymphocytes
Pax6	2	11p					Small eye	Aniridia	CNS, eye, pituitary
Pax7	4	1p							CNS muscle, nose
Pax8	2	2q							CNS, kidney, thyroid
Pax9	12	14q							vertebral column, tooth buds

PD, paired domain; OD, octapeptide domain; HD, homeodomain; CNS, central nervous system