2-5-03

Urogenital Development

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The Origin of the Kidney

In the vertebrate embryo, the first stage of kidney development occurs after gastrulation, within a region called the intermediate mesoderm.

The process of gastrulation turns a single sheet of pluripotent embryonic Ectoderm, or the epiblast, into three primary germ layers.

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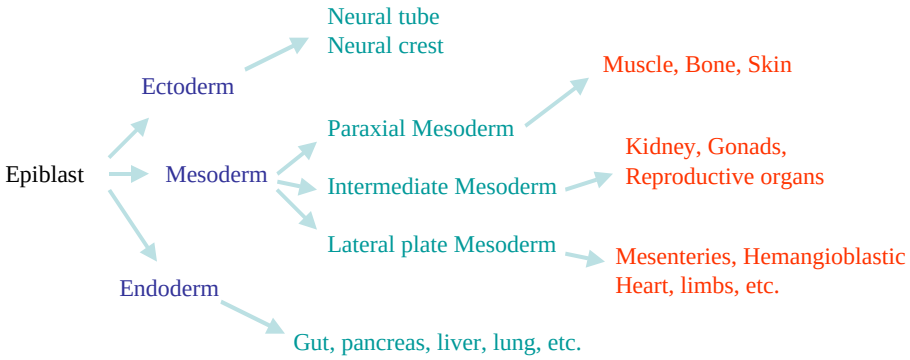
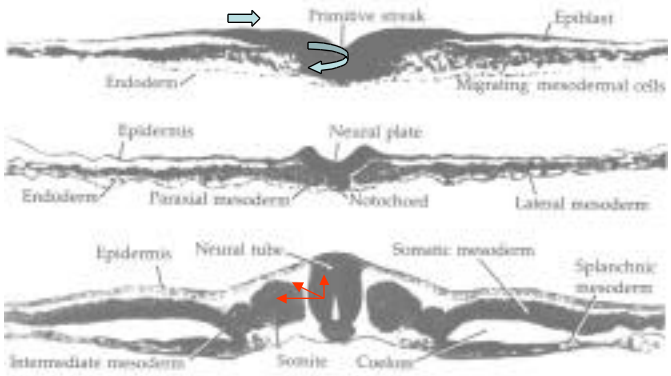


Cross sections through progressive stages of the chick reveal the organization of the early mesoderm

The primitive streak is a furrow through which epiblast cells migrate

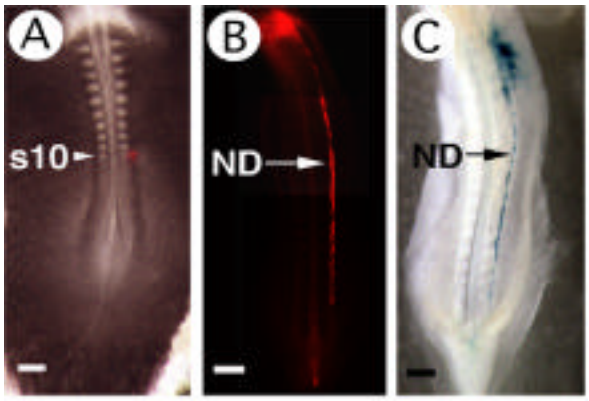
The distance from the neural plate, the embryonic axis or midline, determines the type of mesoderm and its eventual fate



The midline NOTOCHORD secretes factors that organize the dorsal-ventral and medio-lateral axes of the embryo.

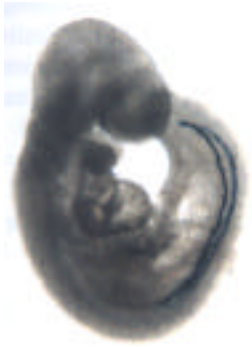
The first epithelial component of the urogenital system is the NEPHRIC DUCT. It arises within the intermediate mesoderm adjacent to the 10-12th somites and extends posteriorly.

DiI lineage tracing in the chick embryo reveals that the Nephric Duct forms by extension rather than by recruitment of mesoderm to the epithelial duct.

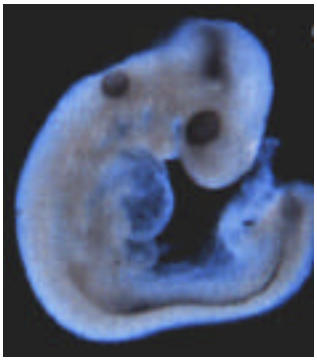


Obara-Ishihara et al., Develop. 126, 1103 (1999)

As the Nephric duct forms and grows caudally, Lim1 and Pax2 expression mark the epithelium of the duct.



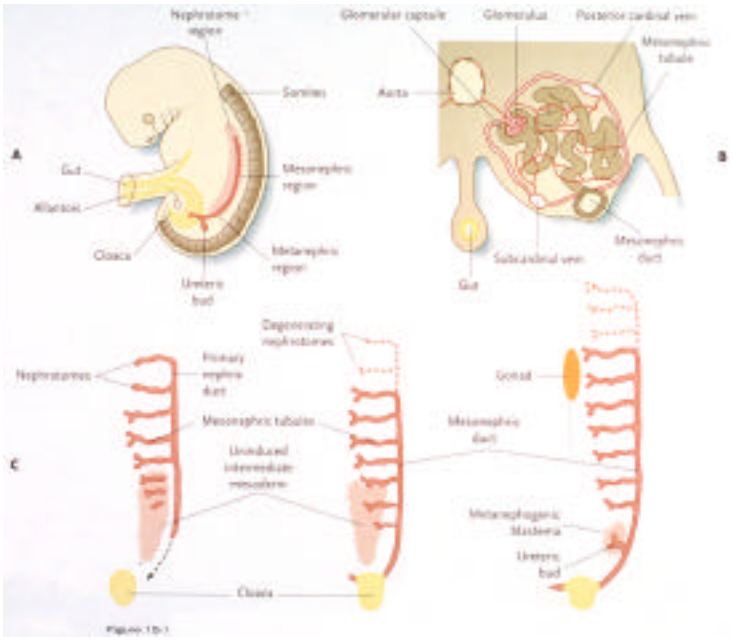
Lim1



Pax2

Lim1 is essential for nephric duct formation
Pax2 and its related gene Pax8 are also essential for nephric duct formation

Overview of early Pro-, Meso-, and Metenaphric patterning

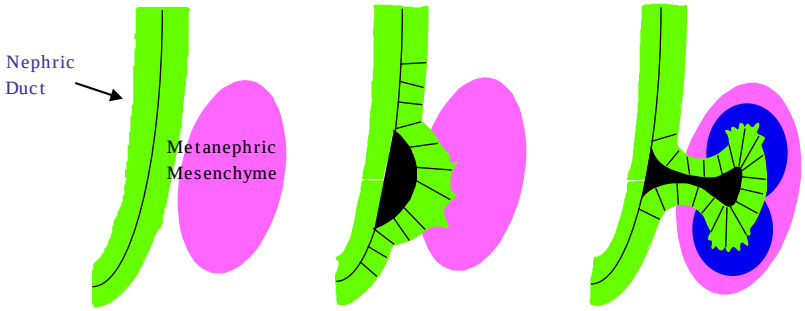


The outgrowth of the Ureteric bud, or metanephric diverticulum is the first critical step in the development of the adult kidney.

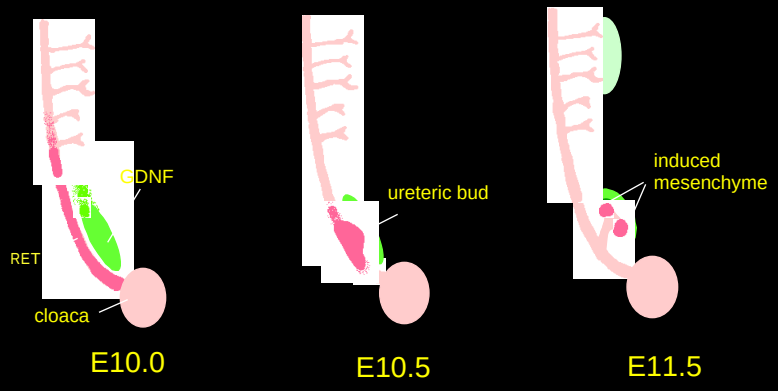
The ureteric bud epithelia and the adjacent metanephric blastema, or metanephric mesenchyme, are the two primordial cell types of the adult kidney.

Reciprical inductive interactions between the ureteric bud epithelia and the metanephric mesenchyme generate the nephrons, collecting ducts and the three dimensional architecture of the kidney.

How does the Ureteric Bud know where and when to grow out?



- Nehric duct epithelium migrates and invades the mesenchyme
- Localized cell migration and proliferation precede branching
- Cells must be motile but retain essential epithelial character

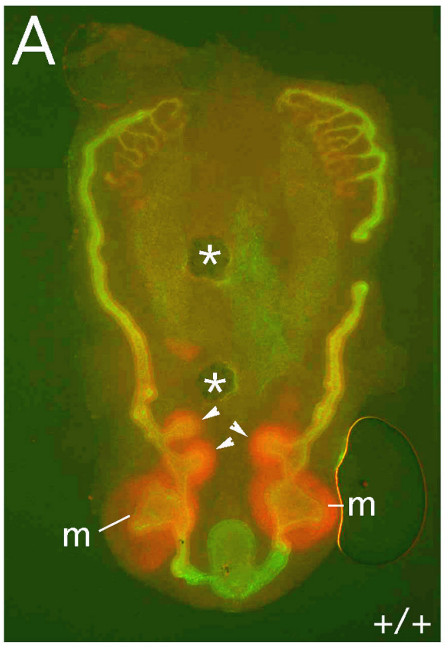


The RET signaling pathway is essential for ureteric bud growth and branching morphogenesis.

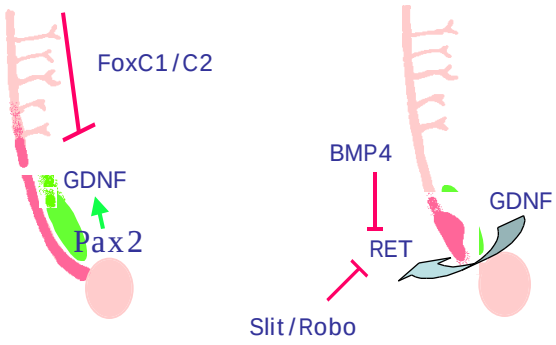
The signaling complex requires RET, GDNF, and GFRα1.

Mouse mutants in any one of these show inhibition of ureteric bud growth.

The GDNF protein is Sufficient to induce Ureteric bud outgrowth Within the posterior Aspect of the nephric Duct.



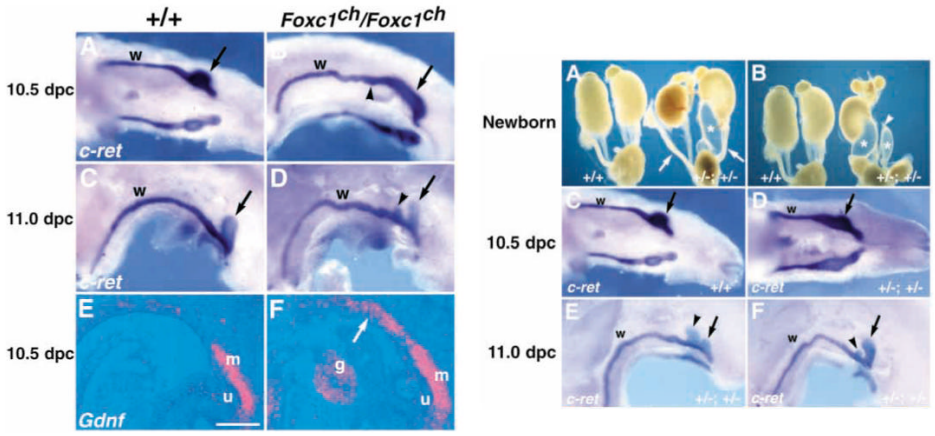
Multiple factors limit RET signaling to the posterior nephric duct



The transcription factor Pax2 activates GDNF, whereas
The transcription factors FoxC1/C2 suppress GDNF expression

The secreted signaling molecule BMP4 suppresses the activity of GDNF

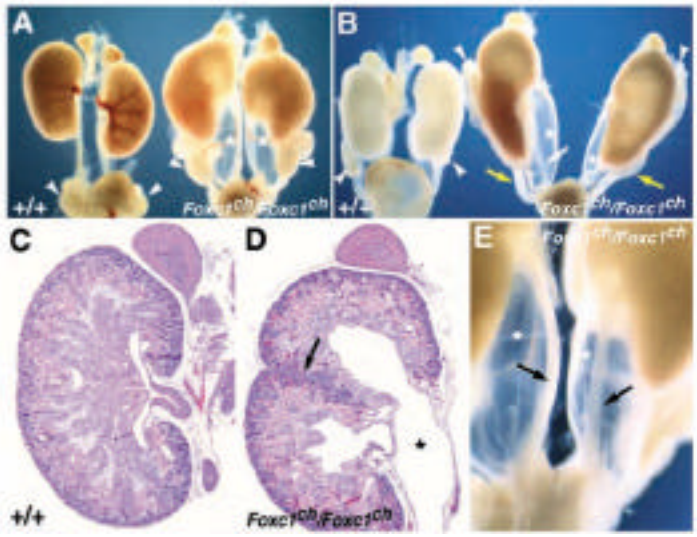
The Foxc1 and Foxc2 genes limit the anterior domain of GDNF expression

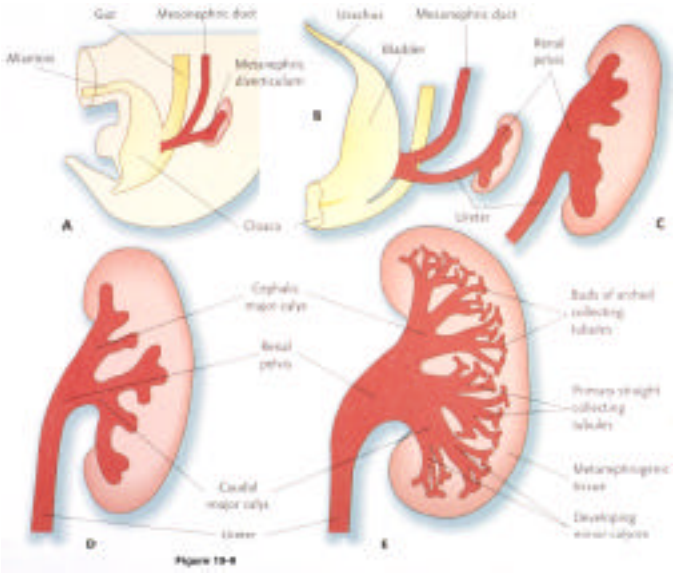


This results in larger or multiple ureteric buds.

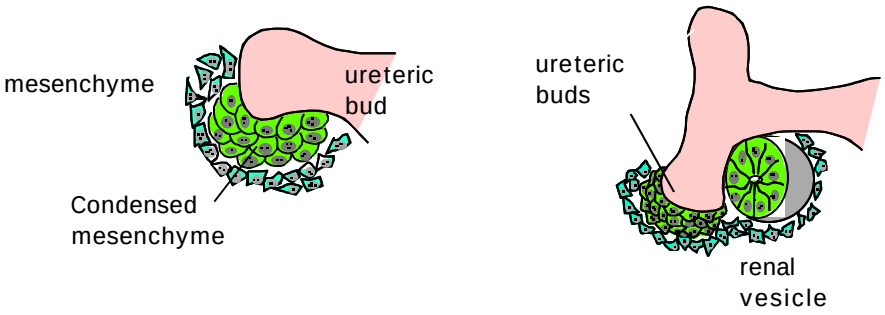
The more anterior, or ectopic, ureteric bud fails to connect properly to the developing bladder and often results in hydroureter.

Hydroureter in newborn mice with mutations in Foxc1



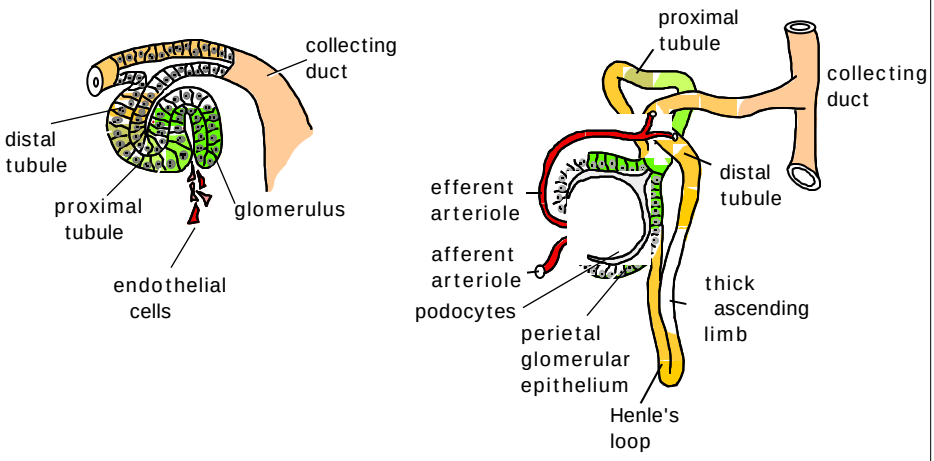


Once the ureteric bud invades the metanephric mesenchyme it undergoes dichotomous branching morphogenesis, this will generate much of the renal papilla



Inductive signals from the ureteric bud induce mesenchyme cells to aggregate and Become a polarized epithelial vesicle.

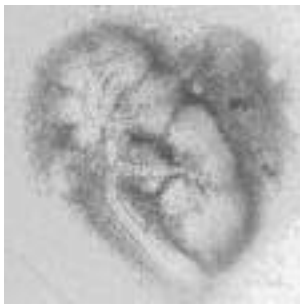
Induction of the metanephric mesenchyme is permissive rather than instructive, i.e. The mesenchyme is already fated to become renal epithelia regardless of the source of induction.



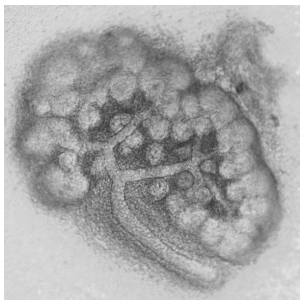
Polarized epithelial vesicles fuse with the branching ureteric bud epithelia, to Form an s-shaped body. The most proximal cleft becomes vascularized by Infiltrating endothelial precursor cells to form the glomerulus.

Kidney Development in Culture

1 day



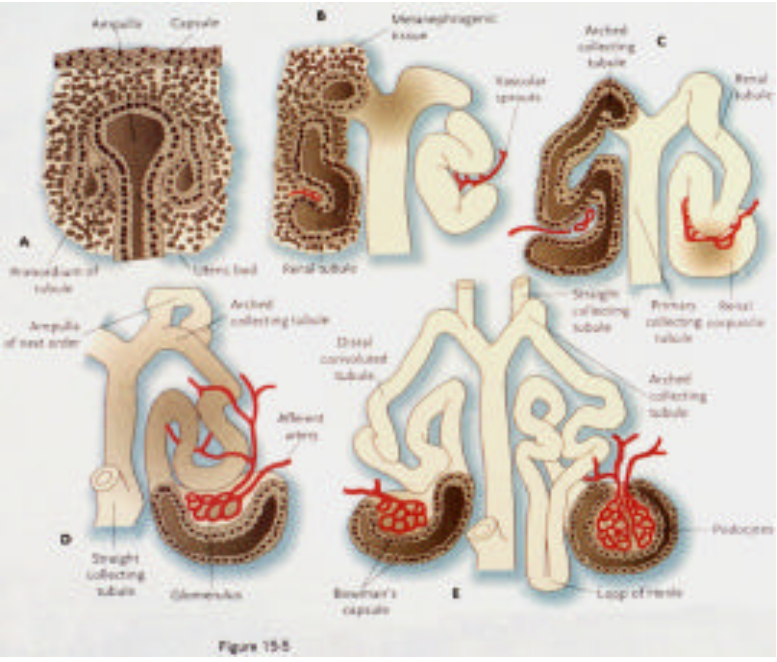
2 days



As the ureteric bud epithelium undergoes branching, it induces a new mesenchymal aggregate at the end of the branched tips. Thus, new nephrons are formed with the oldest being more medullary and the youngest more cortical.

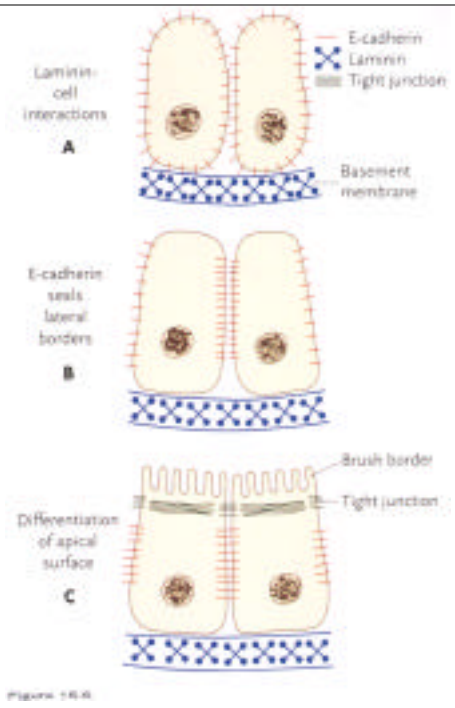
After a series of dichotomous branches, the ureteric bud grows radially and projects a series of single branches to induce the most cortical nephrons in A cascade.

How do induced mesenchmal cells generate nephrons?

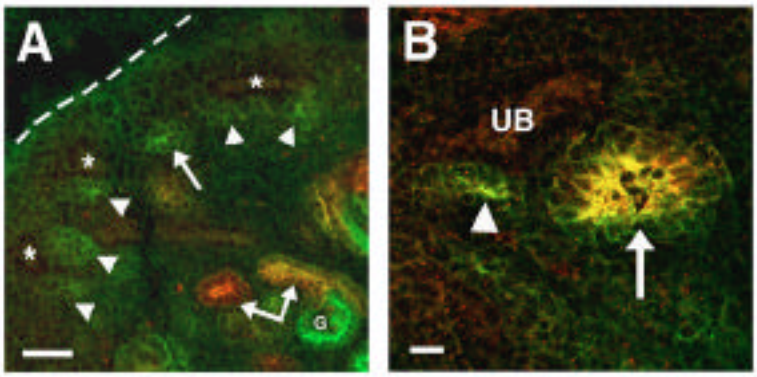


Polarization of the metanephric mesenchyme is a step-wise process that requires changes in cell-adhesion molecule expression, the formation of adherends junctions, the deposition of a laminin containing basement membrane, and the formation of tight junctions.

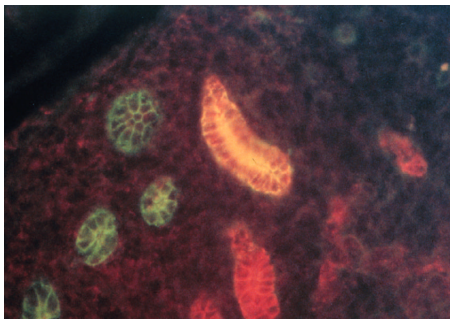
This establishes the apical and baso-lateral sides of the epithelial cell

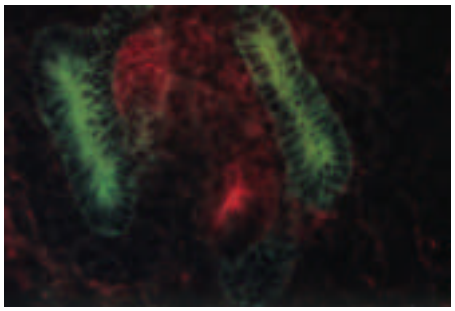


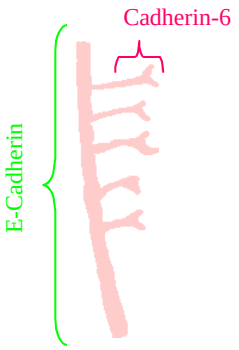
R- Cadherin precedes Cadherin- 6 Induction

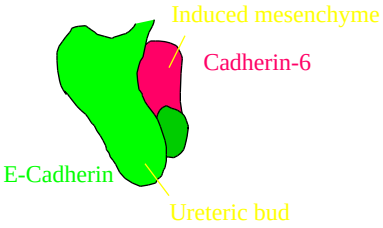


R- Cadherin
Cadherin- 6

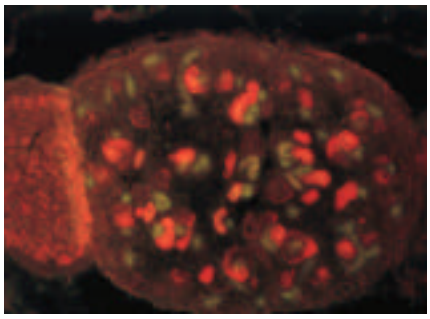


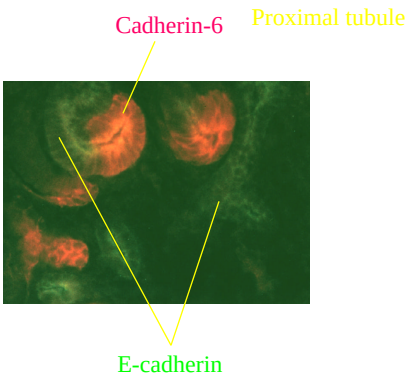




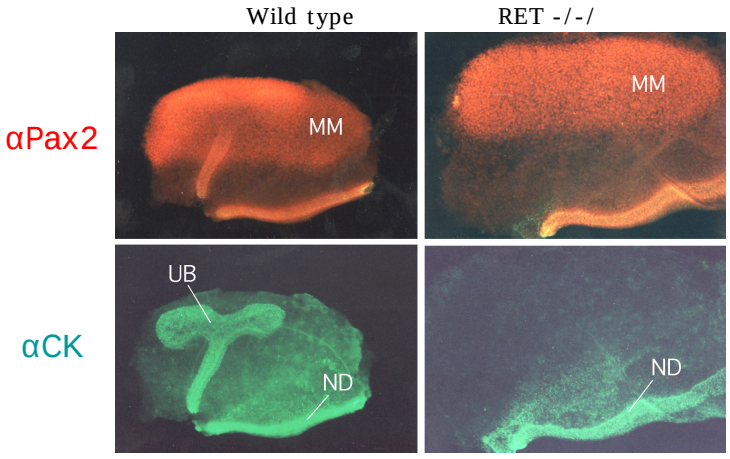


By E14, compartmentalization of the newly formed epithelia is clear. The S-shaped body is demarcated into glomerular, proximal tubule and distal tubule epithelia.



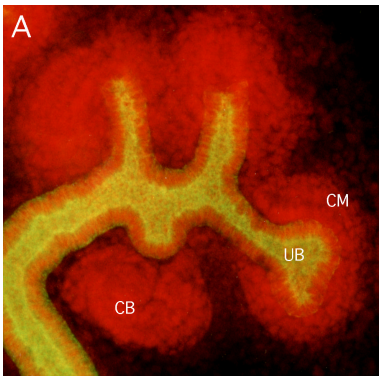


Pax2 Marks the Metanephric Mesenchyme Prior to Induction

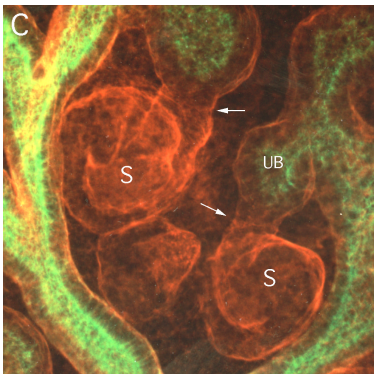


E11.5 whole mount Ab stain

The Pax2 gene is a critical factor in the mesenchyme that controls the Response to inductive signals



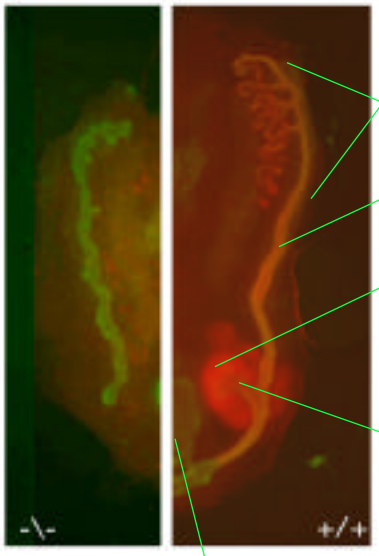
Cytokeratin Pax2



Laminin Cytokeratin

In Pax2 null embryos, the Nephric Duct forms but there are no Pro- or mesonephric Tubules, there is no ureteric bud and there is no metanephros. Pax2 is required for the Mesenchyme to respond to inductive signals.

Antibody staining
Pax is red
Epithelial cytokeratin is green



mesonephros
Nephric duct
Metanephric mesenchyme
Ureteric bud
cloaca

Loss of Pax2 Function:

Mice homozygous for a Pax2 null allele have complete renal agenesis

Humans carrying one mutant Pax2 allele (heterozygotes) exhibit - Renal-Coloboma Syndrome

In both mice and humans, Pax2 is haplo-insufficient*, i.e. a reduction in Gene dosage results in a phenotype of varying penetrance.

Thus, the mutations are dominant because both normal Pax2 alleles are required for normal kidney development

*This is also true for other Pax genes, such as Pax6 (aniridia) in the developing eye

Renal-Coloboma Syndrome

Patients typically exhibit at least these 3 symptoms:

1- Renal hypoplasia - due to reduced proliferation of the mesenchyme derived epithelia during development.

2- Vesicouretral Reflux - most likely due to improper connection of the ureter to the bladder or possibly due to inherent defects in epithelial cells of the mature ureter.

3- Optic Nerve Colobomas - due to failure of the optic fissure to fuse. Expression of Pax2 is observed in part of the optic cup and optic stalk.

Other critical regulators of the Mesenchymal-to-epitheial transition Include:

WT1- Wilms tumor suppressor gene

Wnt-4 - secreted signaling molecule

BMP7 - secreted signal

BF-2 - stromal transcription factor

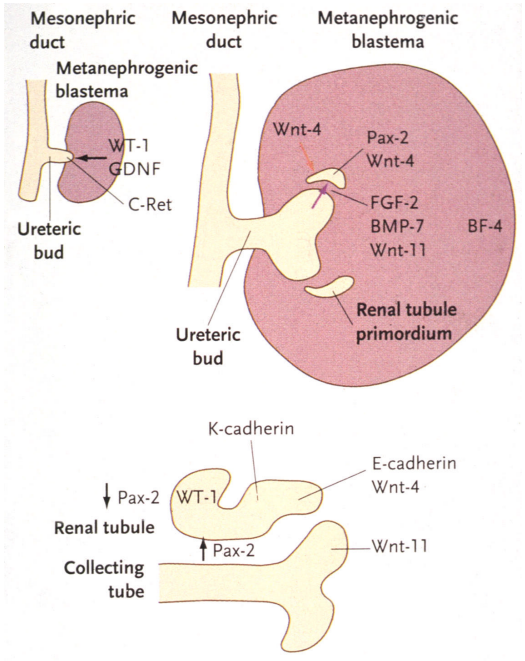
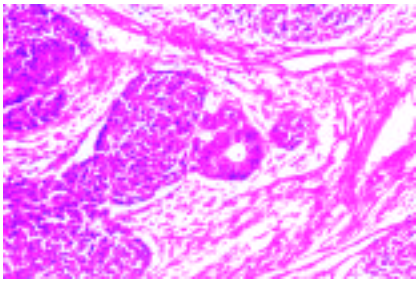
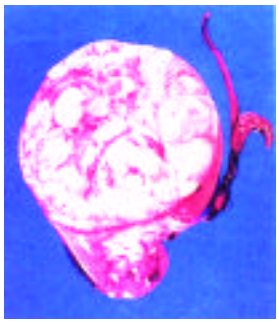


Figure 15-3

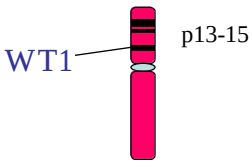
The WT1 gene is associated with Wilms' Tumor

An embryonic kidney tumor that exhibits a triphasic histology
Consisting of blastemal, stromal, and epithelial cell types





Familial Wilms' tumor mapped to a region of chr. 11p13-15

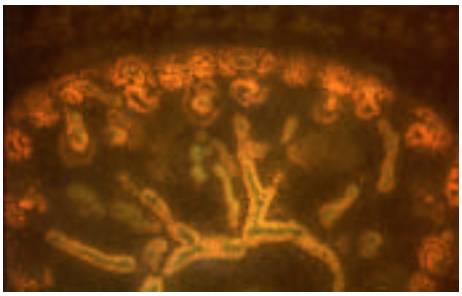


The disease was dominant, i.e. families with a single mutated allele had a significant risk of children with Wilms' tumor.

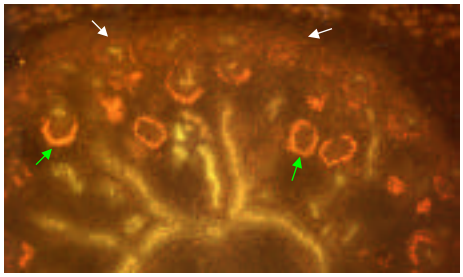
Tumor cells showed a Loss of Heterozygosity (LOH)

Classic two-hit model of tumorogenesis (Knudsen), similar to Rb

Expression of WT1 is dynamic, with low levels of protein in the mesenchyme and increasing amounts in the podocyte precursors of the glomerulus.



Pax2



WT1

PKD

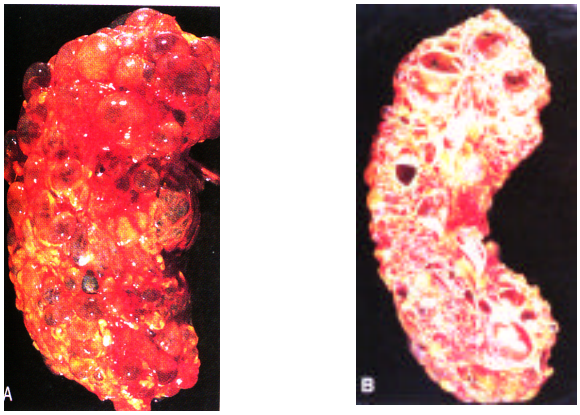
Polystic Kidney Disease (PKD) is the most common single gene Disorder in the adult population. About 1/3000 individuals Have a mutation in the associated genes.

There are dominant and recessive forms of PKD.

The most common is autosomal dominant PKD (ADPKD)

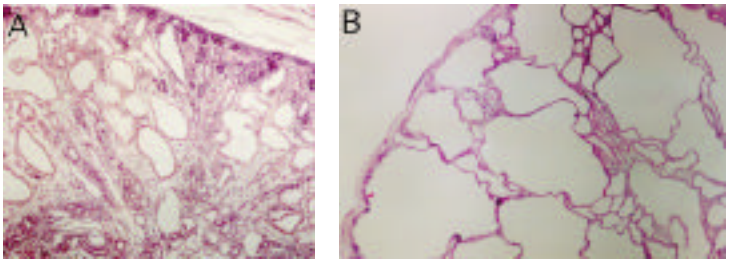
PKD is characterized by the progressive accumulation of renal cysts

Cyst are large fluid filled epithelial spheres that ultimately can displace the normal tissue and lead to end stage renal disease.



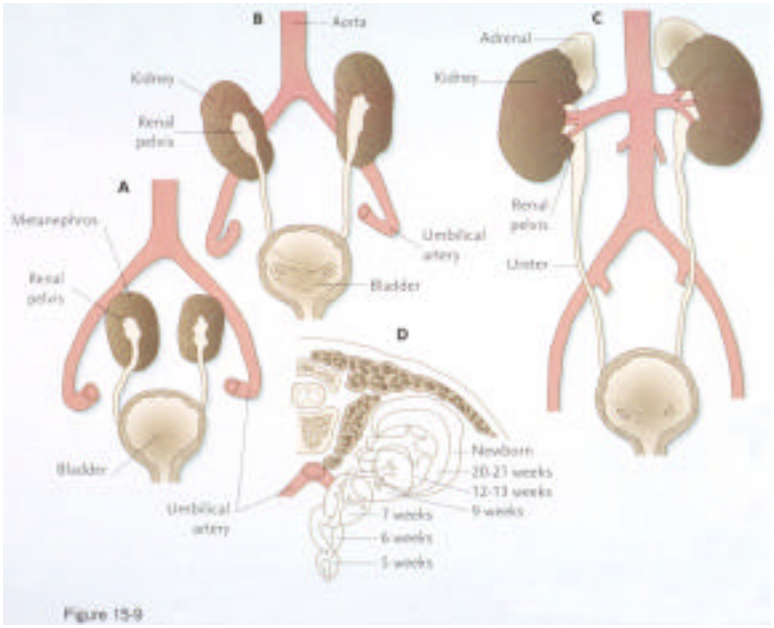
PKD1 null mice get cysts in utero

Thus, polycystin-1 is an essential protein that maintains polarity and/or fluid Transport across the renal epithelia.

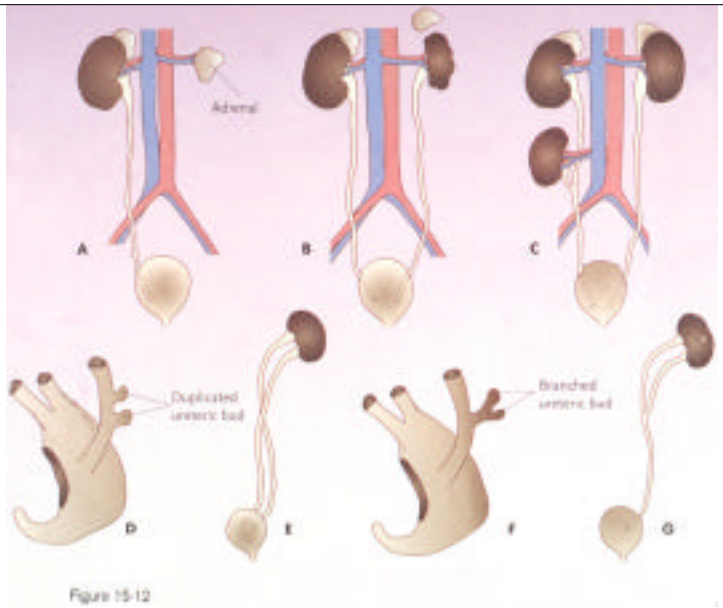


E18 8 days

Heterozygous mice show some cystogenesis later in life, i.e. 18-24 months



Migration of the kidneys from the pelvis



A- Unilateral renal agenesis B- Renal hypoplasia
C- supernumerary (ectopic) kidney

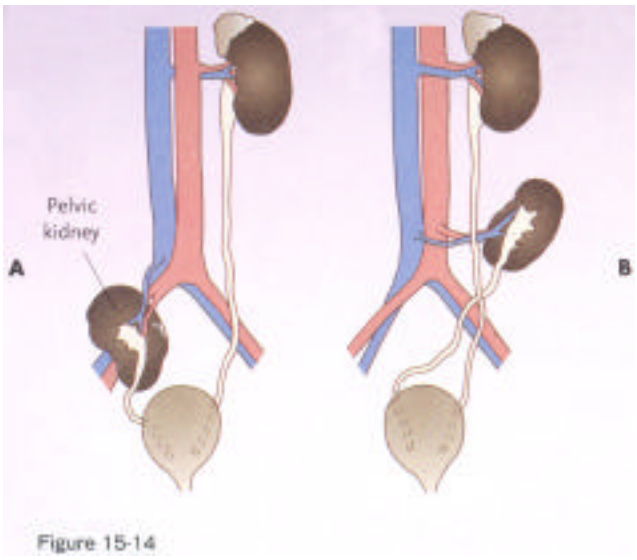


Figure 15-14

A- pelvic kidney

B-crossed ectopia

Horseshoe kidney

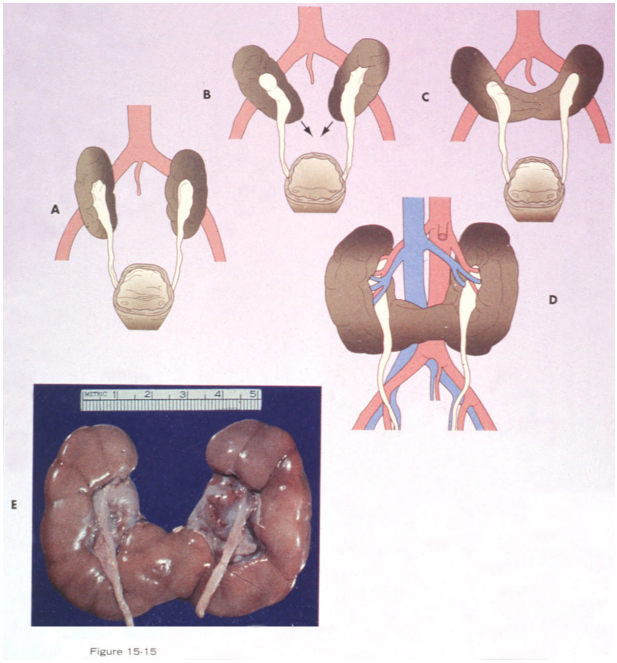


Figure 15-15