

# RENAL DEVELOPMENT

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**SUGGESTED READING:** Larsen, 3rd edition, pp 265 (first three paragraphs) - 266, 268-276 and figure 10-10

## LEARNING OBJECTIVES:

You should be able to:

1. Describe the three kidneys that are produced during development and know what happens to each one.
2. Explain what is meant by 'reciprocal induction' and why it poses problems in interpreting experiments in developing kidney.
3. Describe the stages of nephron formation from the renal vesicle.
4. Discuss the regulators of mesenchymal to epithelial transition in the intermediate mesoderm and metanephric mesenchyme and name three molecules mediating conversion.
5. Describe branching morphogenesis and name the three patterns in the developing metanephros.
6. Discuss three key important ligands and their receptors.
7. Discuss the classification of congenital renal abnormalities that are associated with urological abnormalities and the possible underlying mechanisms for their association.

**SUMMARY:** The urogenital system derives from mesenchymal cells by a process of conversion to epithelia. The development of the kidney relies on three mechanisms of epithelial morphogenesis. 1. Some newborn epithelia migrate extensively (Wolffian duct), 2. some undergo branching morphogenesis (ureteric bud) and 3. some produce highly segmented tubules (nephrons).

## GLOSSARY:

**Angiotensin II:** your favorite vasoconstrictor and regulator of proximal tubule reclamation of NaCl and water by receptor type 1. Receptor type-2 modulates cell growth and seems to play a role in congenital abnormalities.

**Arcade:** a tubule of ureteric bud that induces a few nephrons simultaneously. The nephrons join to a common drainage called a connecting tubule that feeds into the ureteric's collecting duct.

**Cloaca:** common compartment for urinary system and gastrointestinal tract, then divides; precursor of bladder.

**Dipodial:** two feet; **Monopodial:** one foot; branching of the ureteric bud.

**Dysplasia:** qualitative abnormalities; abnormal mesenchymal derivatives.

**Female=Paraophoron, epophoron; Male= ductuli efferentes, epididymis, vas deferens:** epithelial tubules that are descendents of the Wolffian duct, either vestigial in female, or conductive and functional in male.

**Hypoplasia:** quantitative deficiency; fewer, but normal.

**Intermediate mesoderm:** precursor of pro- meso- and metanephric nephrons.

**Lineage marker:** A way to label cells independently of endogenous expression of genes.

Fluorescent dyes or continuously expressed enzymes like  $\beta$ -galactosidase permanently mark cells allowing them and their descendents to be followed irrespective of final state.

**Pronephric duct=Mesonephric duct=Nephric duct=Wolffian duct:** Name changes as the duct is co-opted by a different set of tubules (pronephric, mesonephric, ureteric bud, vas deferens).

**Reciprocal induction:** A circular interaction of two compartments. Part A is necessary for Part B; Part B is necessary for Part A. Part A might control the release of substances from Part B and Part B may control the release of substances from Part A. For example, the ureteric bud expresses **Ret**, a receptor tyrosine kinase essential for branching of the ureteric bud while the mesenchyme expresses **GDNF**, its ligand. **Pax-2** is a transcription factor that is necessary for expression of ret in the ureteric bud and GDNF in the mesenchyme.

**Stephens rule:** ureter draining hypo-dysplastic kidney enters genitourinary system in the wrong place.

**Stromal cells:** interstitial cells of the kidney; the source of fibrosis or scarring in disease states. They are marked by **BF-2** a transcription factor specific to stromal cells.

**Trigone:** region of posterior bladder where Wolffian duct and ureteric bud fuse with the cloaca and make up the posterior wall of the future bladder.

**Weigert-Meyer rule:** For ureter duplication, the upper ureter enters the genito-urinary system in the wrong place and always caudal to the entry of the lower ureter.

**Wnt-4** is a secreted glycoprotein expressed by the mesenchyme that is necessary for epithelial conversion.

**WT-1 (“Wilms tumor-1”)**, a transcription factor that is necessary for mesenchymal survival and conversion.

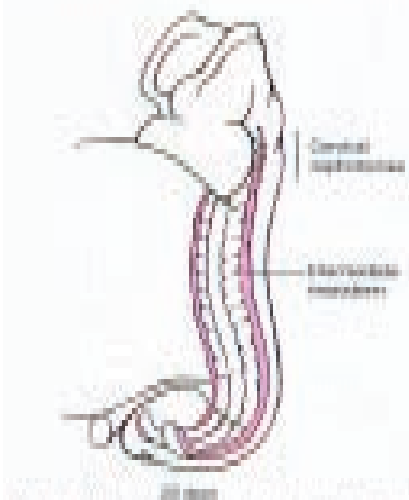


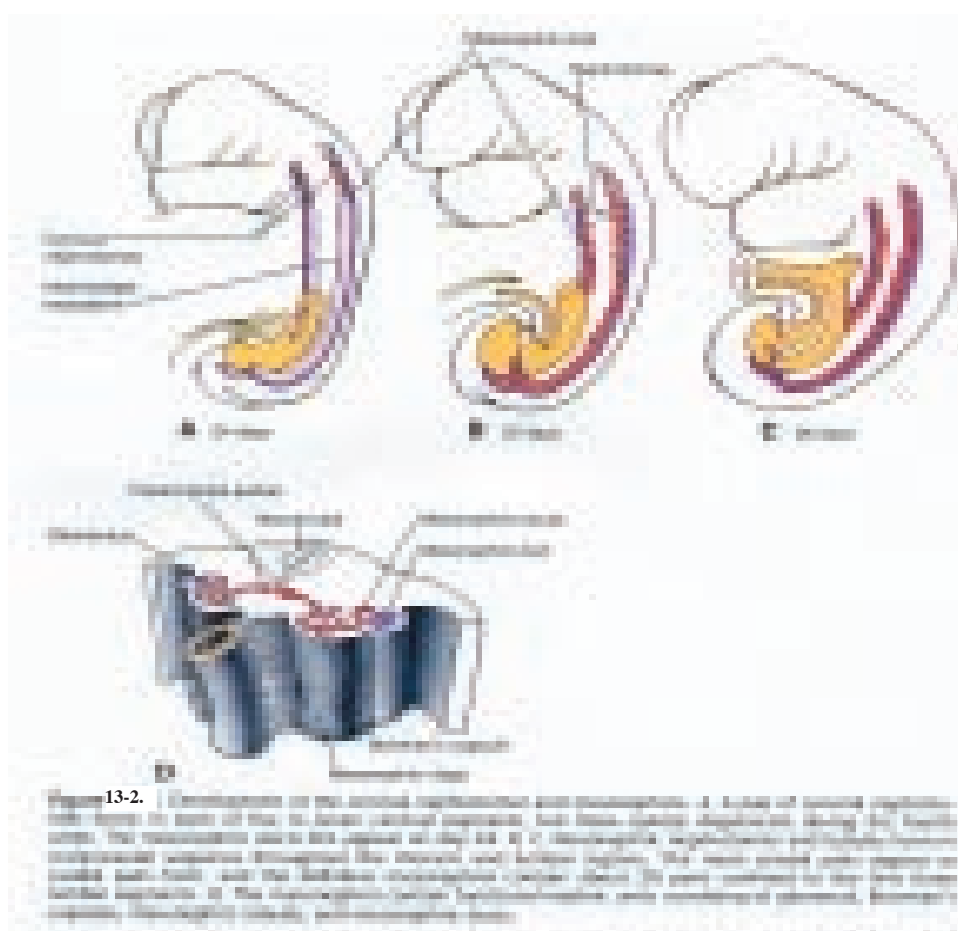
Figure 13-1 The intermediate mesoderm gives rise to paired, segmentally organized mesonephroses from the cervical to the sacral regions. Cervical mesonephroses are visible (arrow) only in the fourth week and are sometimes referred to collectively as the protonephros.

## THE FORMATION OF THE WOLFFIAN DUCT

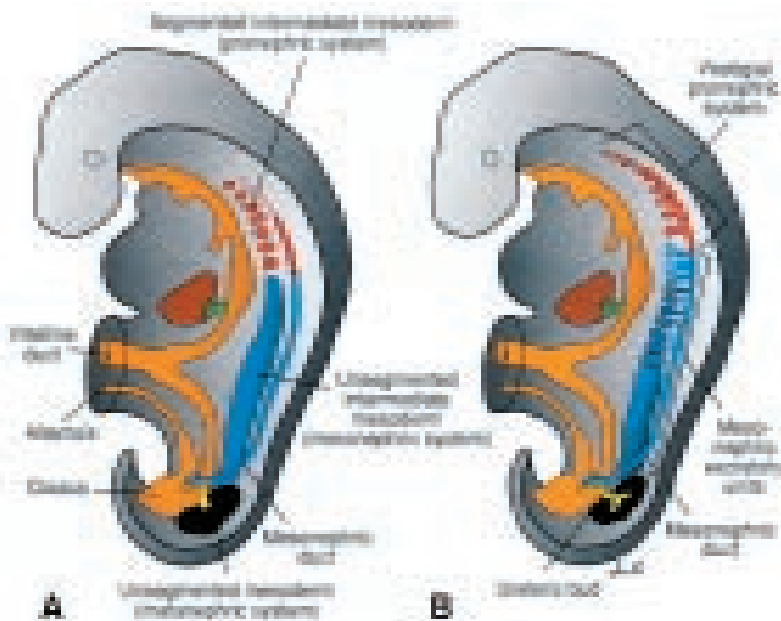
The paired stripes of intermediate mesoderm, situated on either side of the midline between the somites and lateral plate mesoderm, give rise to epithelial cells. These epithelial cells form the ducts of the urogenital system and the nephrons that attach to the ducts.

### 1. Three kidneys in embryonic development: Pronephros, Mesonephros, Metanephros

The **pronephric kidney** forms first from mesenchyme at day 20-23. It is a series of cystic vesicles in the cervical region of the embryo (Figure 13-1). They never form complete nephrons and degenerate by day 25. Before disappearing they form an epithelial lined duct, ‘**mesonephric duct**’. As this duct extends caudally (blue line in Figure 13-2) within the body of the intermediate mesoderm, tubules appear on either side, which form the **mesonephric kidney**. 40 tubules form in a cranial to caudal direction and at the same time they regress in a cranial to caudal direction, leaving an average of 20 at any moment. They look like simplified nephrons (no loop of Henle) with a glomerulus and a tubule conducting filtrate



and emptying into the mesonephric duct, now renamed the **‘Wolffian duct’**. They are active till day 70 and then disappear. In fact, in females, the entire mesonephros and mesonephric/Wolffian duct almost all disappear (see below). However, in both males and females the formation of the Wolffian duct is critical because near its insertion into the **cloaca** (Figure 13-3) (which it finally reaches on day 26), a new tubule system appears, called the **‘ureteric bud’**. The ureteric bud is an invasive

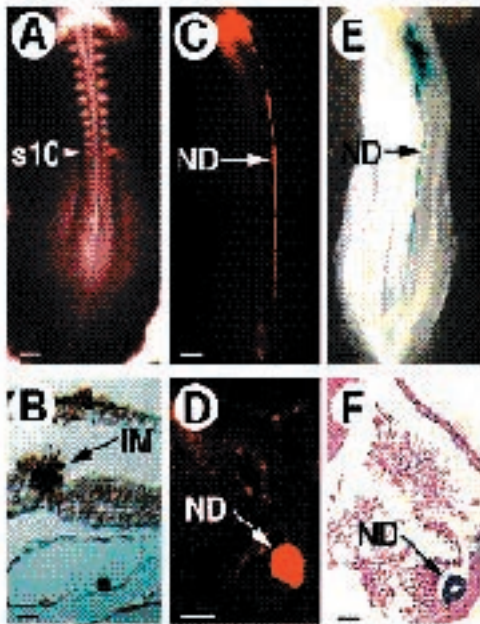


**Figure 13-3.** **A.** Relationship of the intermediate mesoderm of the pronephric, mesonephric, and metanephric systems. In cervical and upper thoracic regions intermediate mesoderm is segmented; in lower thoracic, lumbar, and sacral regions it forms a solid, unsegmented mass of tissue, the nephrogenic cord. Note the longitudinal collecting duct, formed initially by the pronephros but later by the mesonephros. **B.** Excretory tubules of the pronephric and mesonephric systems in a 5-week old embryo.

epithelial tubule which branches into the surrounding intermediate mesenchyme (starting day 28) to form the collecting ducts of the '**metanephric kidney**'. The trunk of the ureteric bud becomes the **ureter**.

## 2. The Wolffian Duct

The mesonephric or Wolffian duct is critical because it is the source of the ureteric bud. Its formation during kidney development is the first instance when mesenchyme converts to epithelia. A patch of intermediate mesenchyme at somite level 10 is the source of cells that will form the duct. This was discovered by use of a **lineage marker**-a fluorescent dye injected into these and no other cells, giving rise to a fluorescent cord of cells (Figure 13-4). The cord forms in a 24 hour period by rapid proliferation and migration of these cells towards the cloaca. The two layers of cells that surround the cord, the surface ectoderm and lateral plate mesoderm, then produce growth factors that convert the cord cells into an epithelial duct. Hence a two step process of Wolffian duct formation, streaming of cells followed epithelialization.



**Figure 13-4.** Wolffian or Nephric duct (ND) derives from cells at somite 10 (s10). This is shown by injection of a red vital dye (A) or  $\beta$ -galactosidase expressing retrovirus (B) in intermediate mesenchyme (IM) next to somite 10, and the production of a duct 24 hours later (C, E). Figure B, D, F are the forming duct seen in cross section. In Figure F we see that the duct is an epithelial tubule.

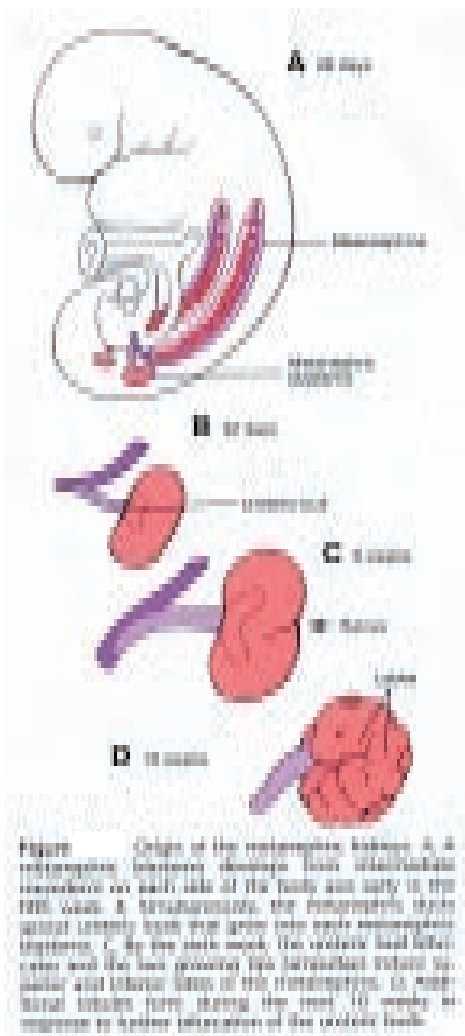
## THE METANEPHRIC KIDNEY

### 1. Ureteric Bud + Metanephric Mesenchyme = Reciprocal Induction

Adult kidney development starts when the **ureteric bud** extends from the Wolffian duct and proceeds to invade the most caudal part of the cord of intermediate mesenchyme called the **metanephric mesenchyme** (Figure 13-5). The bud then branches within the corpus of the mesenchyme (metanephric blastema) to form a complex tree. The metanephric mesenchyme responds to the invading bud by proliferating, then condensing to form coronas of cells around each branch of the bud. Within the condensed mesenchyme, cells convert into epithelial cells. These epithelia form cysts called 'renal vesicles' (see Figure 13-7) that are the precursors of nephrons.

Classical experiments using isolated embryonic kidneys showed that the development of the mesenchyme required the ureteric bud and conversely, branching of the ureteric bud required the metanephric mesenchyme. This is called **reciprocal induction** i.e. the two compartments require each other for development and have been shown to regulate each other's production of important molecules. N.B. Any defect in one compartment causes abnormalities in the other.

### 2. How to Make a Nephron.



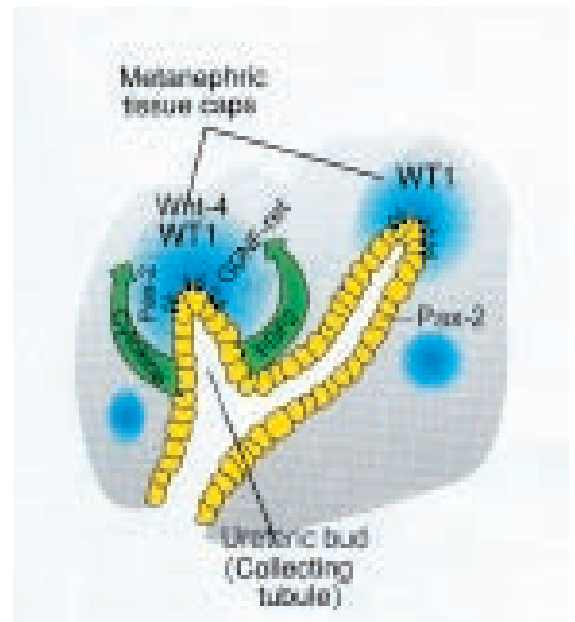
FROM MESENCHYME TO RENAL VESICLE=INDUCTION (Figure 13-6)

- 1) Starting as scattered cells, the mesenchyme condenses at the tips of the ureteric bud to form coronas of cells.
- 2) The coronas express **WT-1** a transcription factor. When WT-1 is mutated it leads to Wilms Tumor, a tumor of the kidney in children; when WT-1 is deleted it leads to agenesis of the kidney because the mesenchyme dies. Hence WT-1 is essential for mesenchymal survival.
- 3) The ureteric bud and the coronas express **Pax-2** a second transcription factor. When Pax-2 is mutated there are defects in eye and kidney formation; when Pax-2 is deleted there is no ureteric bud or metanephric mesenchyme. This is because Pax-2 is expressed in both the ureteric bud and the metanephric mesenchyme where it controls one part of reciprocal induction (see below).
- 4) Clusters of Pax-2, WT-1 cells then express **Wnt-4**. Wnt proteins are secreted glycoproteins that regulate developmental choices. It is thought that the ureteric bud secretes a factor that induces Wnt-4.
- 5) It is currently believed that once Wnt-4 and Pax-2 and WT-1 are expressed by the mesenchyme, the ureteric bud has done its job, and is not needed further. **Conclusion:** Invasion of the ureteric bud causes a cascade of events: Proliferation>> upregulation of WT-1 and Pax-2>> expression of

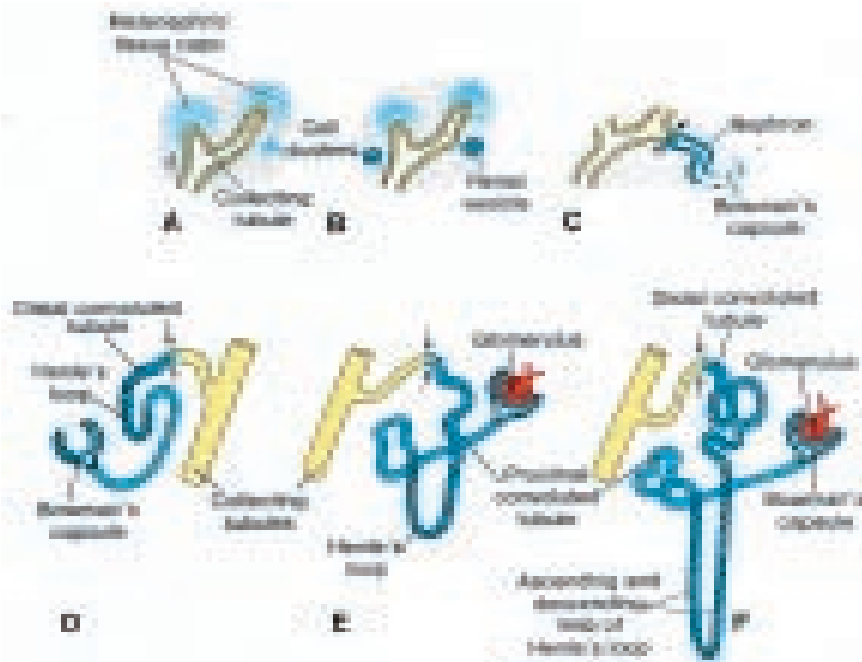
Wnt-4>>nephrogenesis.

FROM RENAL VESICLE TO NEPHRON (Figure 13-7)

The renal vesicle (Figures 13-7 and 13-8) and its descendant—the C-shaped body (Figure 13-8, panel C) are already segmented nephrons. The cells will form the distal convoluted tubule. The steps are



**Figure 13-6.** Genes involved in differentiation of the kidney. WT1, expressed by the mesenchyme, enables this tissue to respond to induction by the ureteric bud. GDNF, also produced by the mesenchyme, interacts through its receptor RET in the ureteric bud epithelium to stimulate growth of the bud and maintain the interactions. The growth factor FGF2 and a cytokine stimulate proliferation of the mesenchyme and maintain WT1 expression. Wnt-4 is then produced by clusters of cells producing Pax-2 and WT1. Pax-2 is expressed by the ureteric bud as well.



**Figure 13-7.** Development of a metanephric excretory unit. *Arrows*, the place where the excretory unit (blue) establishes an open communication with the collecting system (yellow), allowing flow of urine from the glomerulus into the collecting ducts.

Renal vesicle—Comma Shaped Body—S-Shaped Body—Nephron. Note that the endothelial cells invade at the glomerular cleft (panel B). These endothelial cells will contact the visceral epithelial cells, i.e., the **podocytes** in the S-shaped body and form the glomerular basement membrane (GBM). Note that Bowman's space is continuous with the lumen of the proximal tubule (panel D).

FUSION: The nephron develops in physical separation from the ureteric bud. How does the distal tubule of the nephron fuse with the collecting duct? Both express E-cadherin (this cadherin causes homotypic interactions) and perhaps this participates in the fusion.

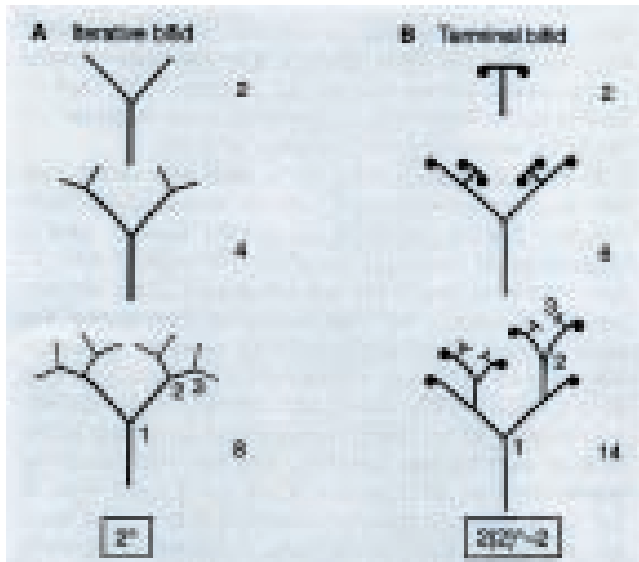
### 3. How to Make a Collecting System from a Ureteric Bud.

**Pattern of Branching:** The initial branching is bifid or **dipodial**. The subsequent branch is a single or **monopodial** which is derived from the cortical side of the initial branches. This branch then repeats bifid branching. Thus there is an alternation of dipodial and monopodial branching which is necessary because one of the



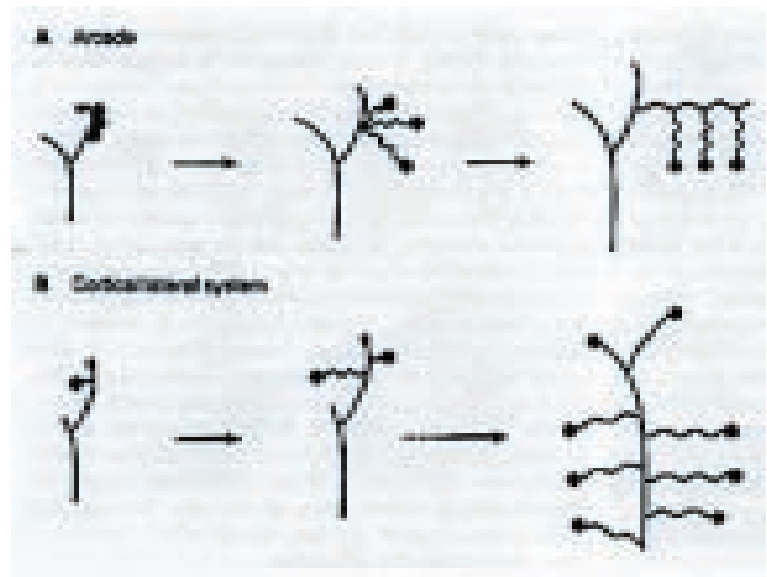
**Figure 13-8.** The progression of cells at the tips of the ureteric bud into the nephrons. A. Scattered mesenchymal cells. B. Condensed Pax-2, WT-1, Wnt-4 expressing cells at the tips of the ureteric bud. C. Reorganization of these cells into epithelia, and the formation of a segmented tubule. The elongated pole facing the ureteric bud is the distal convoluted tubule. D. Another segment forms the future glomerulus. E, F. Invasion of capillary

two bifid branches will always fuse with a nephron and be removed from subsequent branching morphogenesis (Figure 13-9). This reiterative pattern occurs 15 times in the human kidney, producing 65,534 branches with nephrons. Then after the 15<sup>th</sup> division, a new process occurs, called **arcade** formation (Figure 13-10). Here three nephrons are induced along the trunk of a single branch and will all feed into that branch. Finally the tips of the ureteric tree progress directly towards the capsule, inducing 10 **lateral** nephrons for each collecting duct. The combined effect of



**Figure 13-9.**  $2^n$  type branching of the ureteric bud, called dipodial, is wrong. The correct pattern is  $2(2^n)-2$  shown on the right and can be explained as dipodial alternating with monopodial.

**Figure 13-10.** Arcades and lateral branching patterns in the kidney. (A) Arcades develop by simultaneous induction of several nephrons whose connecting tubules fuse and then elongate to form the arcade. (B) The final branch continues to induce as it elongates towards the cortex generating laterally situated nephrons.



bifid, lateral, and arcade formation gives us one million nephrons.

What regulates branching? It has been known for many years that molecules produced by mesenchyme regulate the structure of the ureteric bud. The most important one is called Glial Cell Line Derived Neurotrophic Factor (**GDNF**). As you would expect, the ureteric bud tips expresses the GDNF receptor called **RET** (Figure 13-6).

How does GDNF-ret signaling cause ureteric bud growth and branching? Some people think that it acts like a chemoattractant, others say it's a proliferative agent for the ureteric bud tips, others

suggest that it regulates basement membrane of the ureteric bud.

What regulates *ret* expression in the ureteric bud? The expression of *ret* by the ureteric bud depends on signaling from the renal stroma. These signals have not been identified, but they are under control of retinoic acid. This is particularly striking because it shows that stroma, which lies outside the mesenchyme signals to the ureteric bud. Pax-2 in the ureteric bud also controls the expression of *ret*.

What regulates **GDNF** in the mesenchyme—Pax-2! This shows that branching of the ureteric bud and conversion of the mesenchyme are regulated by a common regulator—Pax-2. This is why the knockout of Pax-2 lacks both ureteric bud and mesenchyme. Review these ideas carefully.

### THE CASE OF THE DISAPPEARING MESONEPHROS (Details will also be discussed during Lecture 14.)

What happens to the mesonephric (Wolffian) duct and tubules while the ureteric bud and the metanephric mesenchyme develop into a kidney? It depends on the sex of the embryo (Figure 13-11). In males some of the mesonephric tubules associate with the gonad, and form the head of the epididymis, and the ductuli efferentes, which export sperm from the seminiferous tubules of the testes into the epididymis. In other words, the Wolffian duct changes its function and becomes the conduit for sperm from the testes all the way to the base of the bladder. It is now renamed (yet again) the **vas deferens**. In contrast, the mesonephric tubules do not survive in the female and the entire duct system, including the Wolffian duct vanishes leaving only remnants in the space next to the forming ovary—the epoophoron, and directly next to the base of the bladder—Gartner's cysts).

### THE OTHER END—BLADDER FORMATION

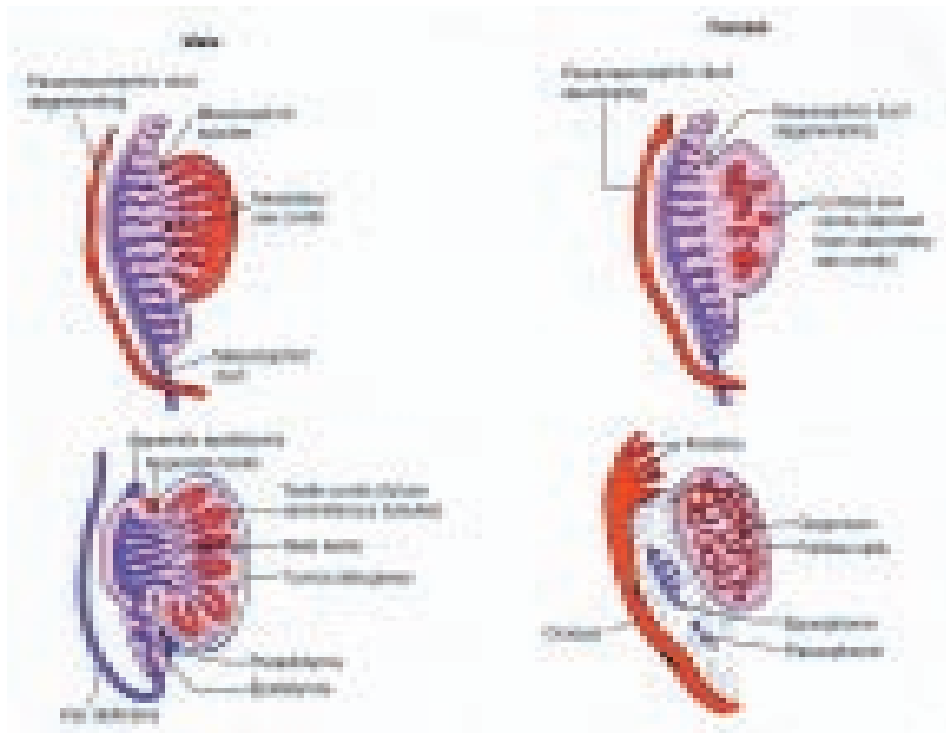


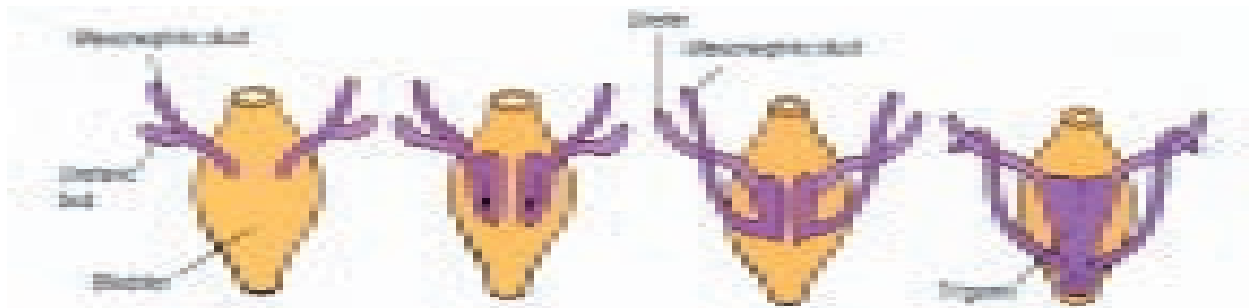
Figure 13-11.



What happens to the caudal end of the Wolffian duct and ureteric bud (the ureter)? The duct systems fuse with the posterior wall of the bladder, a process called **exstrophy**. This occurs by the migration of the mesonephric ducts along the posterior bladder wall, and then the opening of the tubule into a sheet of cells. This Wolffian derived sheet of cells forms the **trigone** (Figure 13-12). Finally the mesonephric ducts (now called the vas deferens) open at the base of the bladder and the ureters open at the top of the bladder.

### THE ASCENT OF THE KIDNEY

One of the strangest and least understood events is how the kidney gets to its final site in the



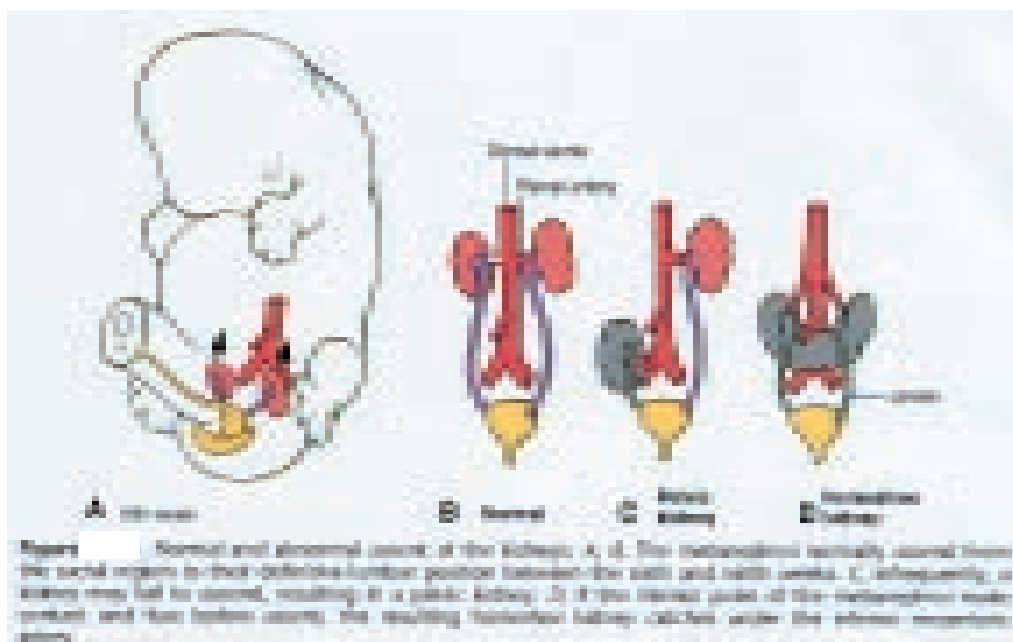
**Figure 13-12.** Exstrophy of the mesonephric ducts and ureters into the bladder wall. Between weeks 4 and 6, the ends of the mesonephric ducts outpocket into the posterior wall of the developing bladder. This process brings the openings of the ureters back into the bladder wall, whereas the opening of the mesonephric duct is carried anteriorly to the level of the pelvic urethra. The triangular region of ectodermal mesonephric duct incorporated into the posterior bladder wall forms the trigone of the bladder.

embryo. The kidney forms at the level of the iliac arteries (Figure 13-13), but its final site is above the pelvic brim. The kidney appears to ascend from the pelvis and in doing so its blood is resupplied by new sprouts from the aorta. Failure to ascend results in a pelvic kidney and could result from fusion of the mesenchyme across the midline (horseshoe kidney). The kidneys might be entrapped below the inferior mesenteric arteries. These kidneys are subject to ascending infection.

### CONGENITAL ABNORMALITIES OF THE METANEPHRIC KIDNEY

#### 1. Importance

In



**Figure 13-13.** Normal and abnormal ascent of the kidney. A, B. The metanephros normally ascends from the sacral region to their definitive lumbar position between the sixth and ninth weeks. C, infrequently, a kidney may fail to ascend, resulting in a pelvic kidney. D, if the normal ascent of the metanephros is obstructed and the kidney cannot cross the resulting fibrous bands called the inferior mesenteric artery.

dysplastic kidneys (a congenital disorder of nephron induction) the mesenchyme converts to

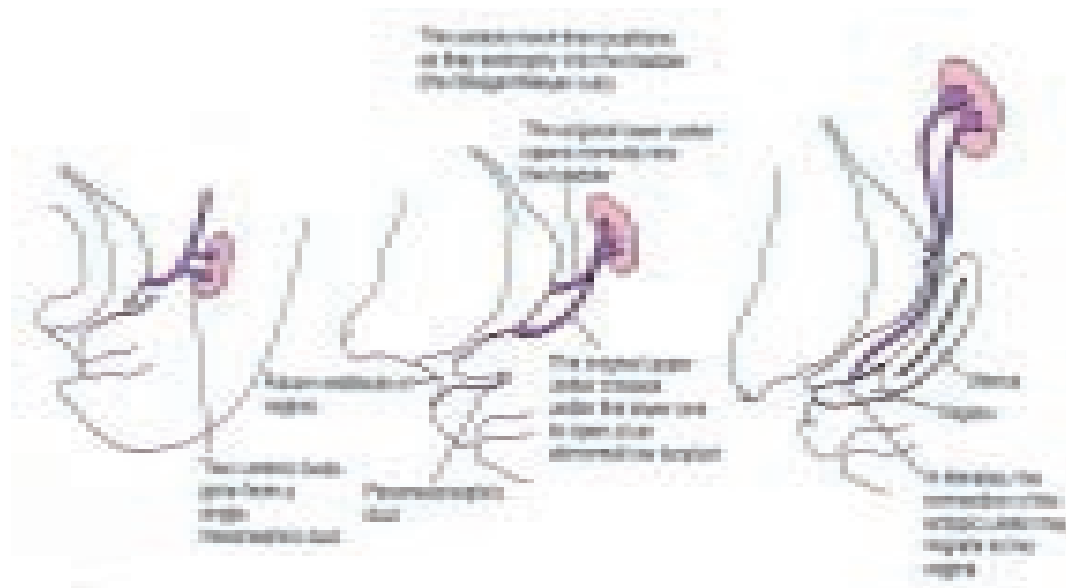
smooth muscle, bone and cartilage rather than epithelia. In hypoplastic kidneys there are fewer nephrons, but with normal architecture (1% of the population has unilateral hypoplasia; there is a 4 fold variation in nephron number in the ‘normal’ population). The Brenner Hypothesis says that variation in nephron number is the cause of hypertension (affects 50 million Americans) and is a renal disease.

## 2. Stephens’ Hypothesis

Both renal hypoplasia and dysplasia are often associated with ureteral abnormalities (the location of the ureter’s insertion into the bladder or lower urinary tracts) (Figure 13-14). Stephens hypothesized that the association of renal and ureteral abnormalities occurs because the origin and trajectory of the ureteric bud is abnormal. Thus, the ureter does not contact the correct piece of intermediate mesoderm (it contacts cells other than metanephric mesenchyme) resulting in absent or reduced tubulogenesis. Second, the ureter, having originated incorrectly, eventually fuses/enters the bladder at the wrong place and the normal tunneling of the ureter through the bladder wall is missing. This tunneling is very important because contraction of the bladder should compress the ureter to prevent backflux and transmission of high pressures to the kidney. The abnormal course of the ureter may also result in kinking, angulation and obstruction. Hence, dysplastic kidneys and obstruction of the ureter is part of an anatomical syndrome.

## 3. Angiotensin II Receptor 2: A molecular basis of the Stephens’ Hypothesis?

Angiotensin II is an important regulator of blood pressure. It causes vasoconstriction by binding to the angiotensin II receptor 1. The function of angiotensin II receptor 2 is less clear, but

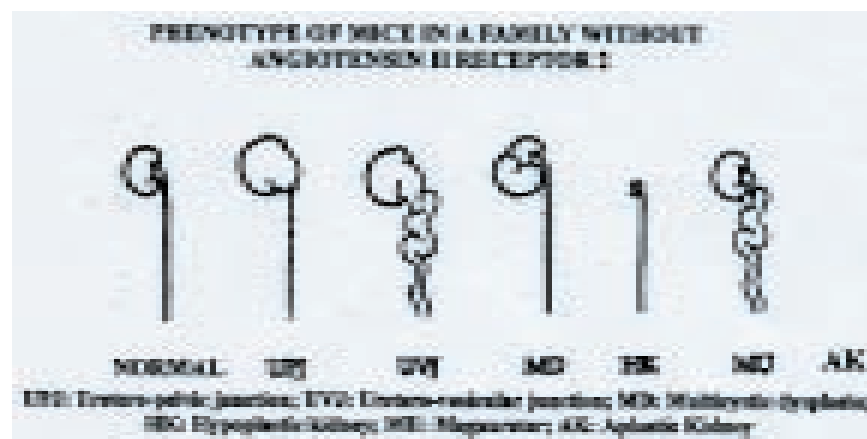


**Figure 13-14.** Ectopic ureter. An ectopic ureter forms from an anomalous “extra” ureteric bud. The mechanisms of formation of the trigone and placement of the vas deferens and ureters on the posterior wall of the primitive urogenital sinus were largely deduced from the Weigert-Meyer rule (see Glossary).

it appears to induce apoptosis. Around the developing metanephric mesenchyme are layers of cells that express the angiotensin II receptor 2. During development, these cells undergo apoptosis and the knockout of the receptor apparently decreases apoptosis of these cells and consequently distorts the trajectory of the ureter, preventing appropriate contact with mesenchyme as well as appropriate migration and fusion of the Wolffian Duct/ ureteric bud with the bladder. As a result the animals have hypoplasia or dysplasia of the kidney, kinked ureters with obstruction at the UPJ (ureteropelvic junction) or UVJ (ureterovesicular junction), ureteral duplications which obey the Weigert-Meyer rule (Figure 13-15) and Stephens rule (ureters draining dysplastic kidneys are ectopic in their insertions). In humans screening of the angiotensin II Receptor 2 gene showed a single base pair change in 70% of patients with hypoplasia-dysplasia-urinary abnormalities which produces no functional receptor (30% of normals have this too). Hence angiotensin II receptor 2 is one modifier of urogenital development. Drugs that block the production of angiotensin 2 (common blood pressure medications—ACEI) can not be given in pregnancy for this reason!

#### 4. Ret Again

Recall that ret is under control of retinoic acid dependent signals from the renal stroma. In the lower urinary tract ret is also under control of retinoic signaling through local stroma. Ret at this location mediates spreading of the Wolffian duct to form the trigone of the bladder



**Figure 13-15.** Urogenital system of mice lacking AII R2

(exstrophy). The importance of these findings is that they demonstrate that a complex signaling pathway- composed of stromal cells (retinoid dependent signals) and Wolffian duct/ureteric bud (ret), is used for epithelial growth in two different settings (branching morphogenesis in the kidney and spreading morphogenesis in the trigone of the bladder). Hence while the Stephen's hypothesis-ATII is an anatomical hypothesis, the finding that retinoid-ret defects generate both urinary and kidney abnormalities shows that the two are linked by using the same signaling pathway.