Part I. Kidney development
Part II. UGT development

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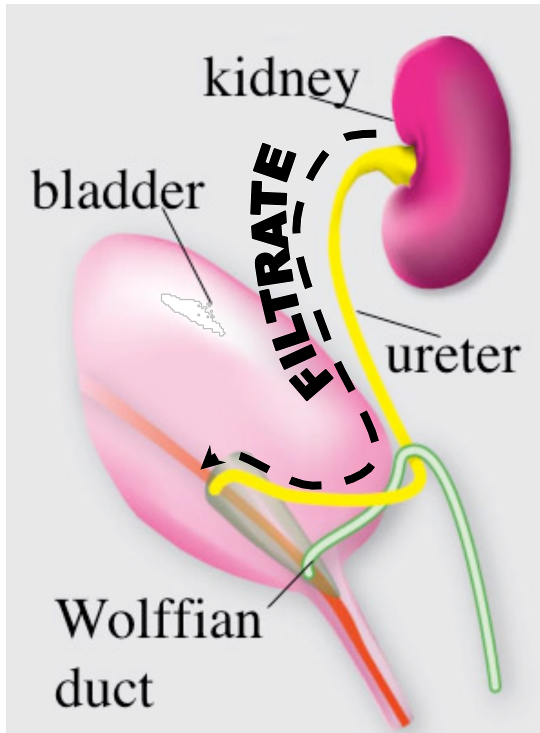
Human Development
Spring 2009

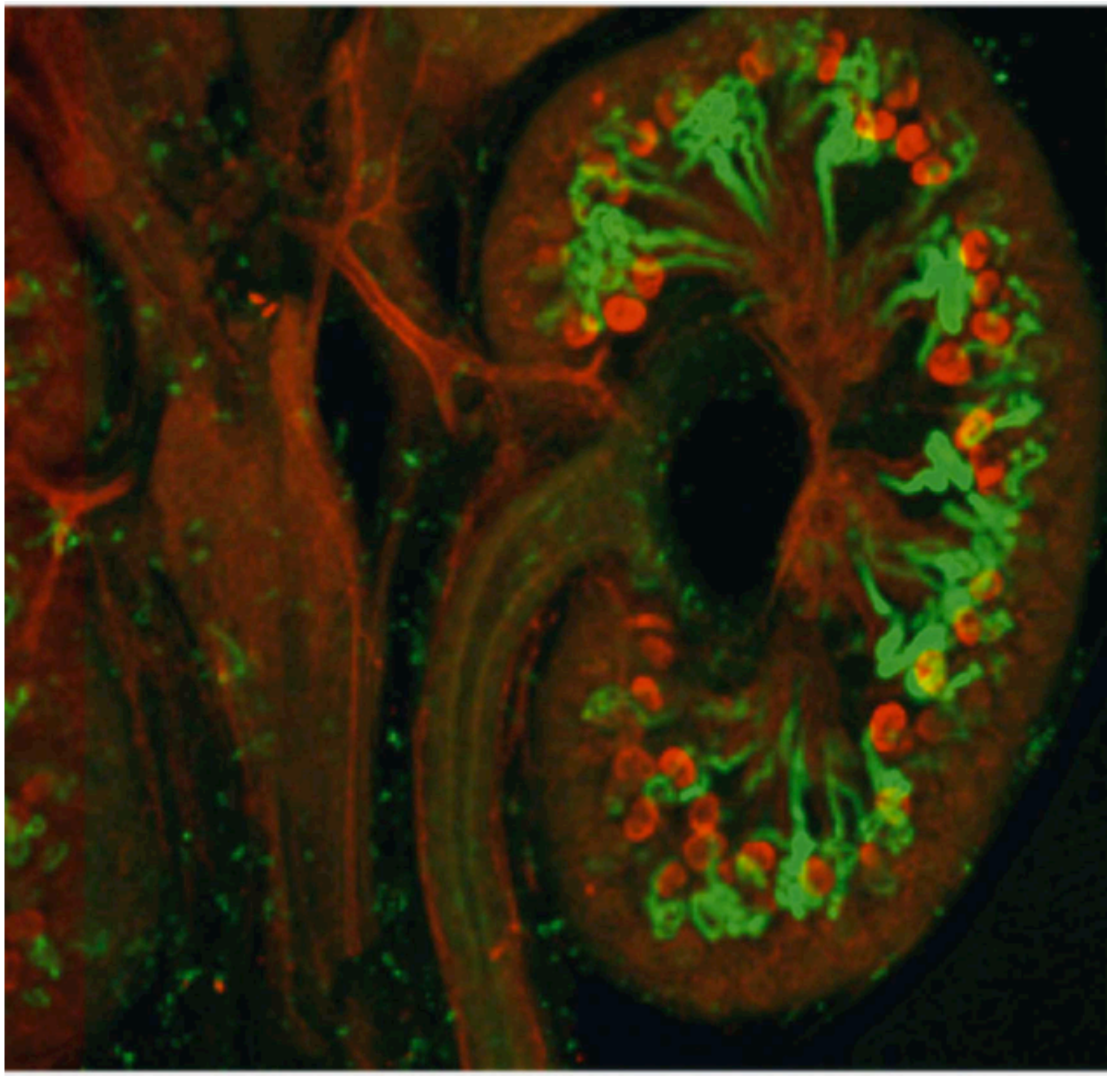


nephrons in the kidney generate urine that is propelled to the ureters and then to the bladder for storage and excretion

The Urinary outflow tract:

- **monitors and regulates extra-cellular fluids**
- **excretes harmful substances in urine, including nitrogenous wastes (urea)**
- **returns useful substances to bloodstream**
- **maintain balance of water, electrolytes (salts), acids, and pH in the body fluids**

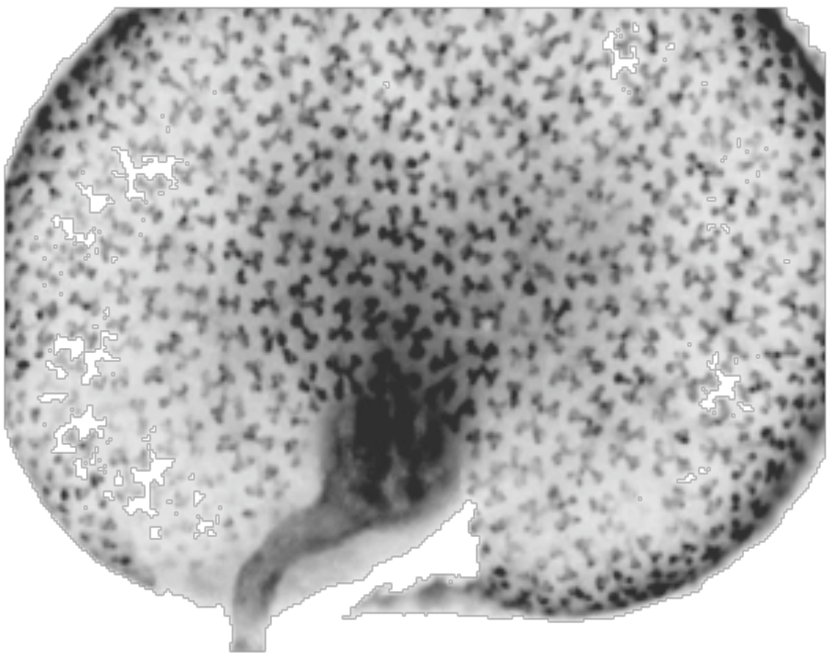
The human kidney has about 500,000 nephrons that filter and modify the blood



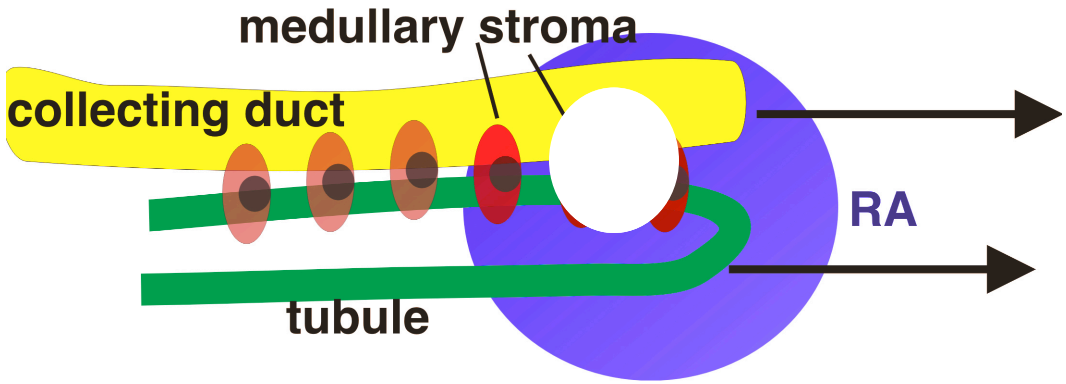
glomeruli

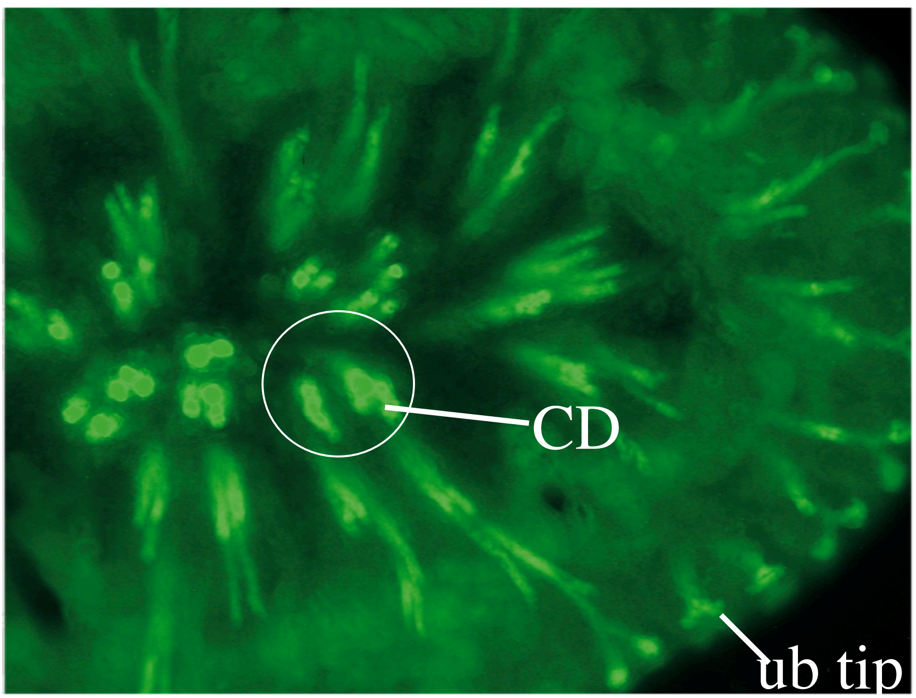
tubules

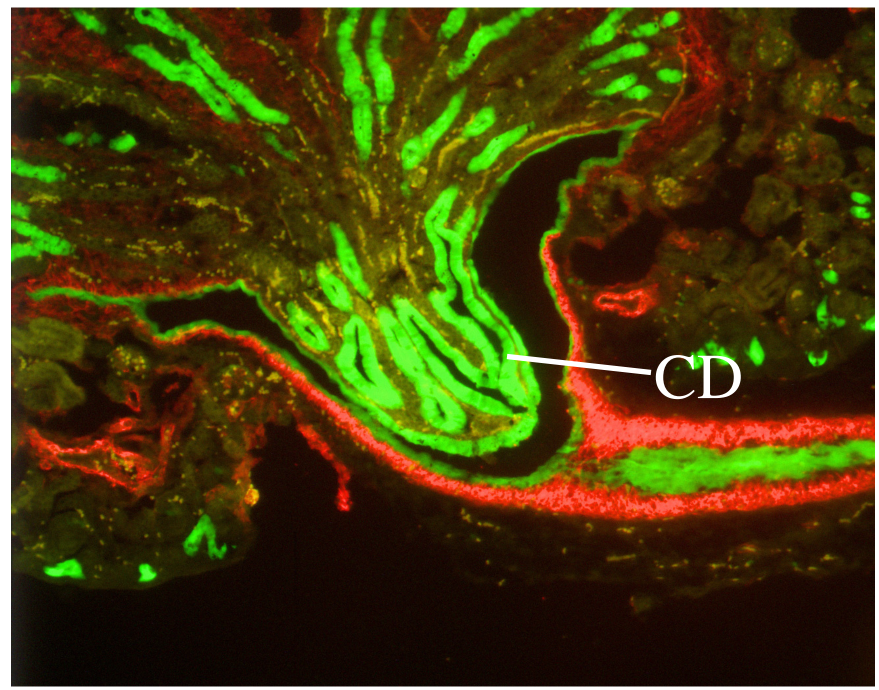
At birth the collecting duct system has tens of thousands of branches



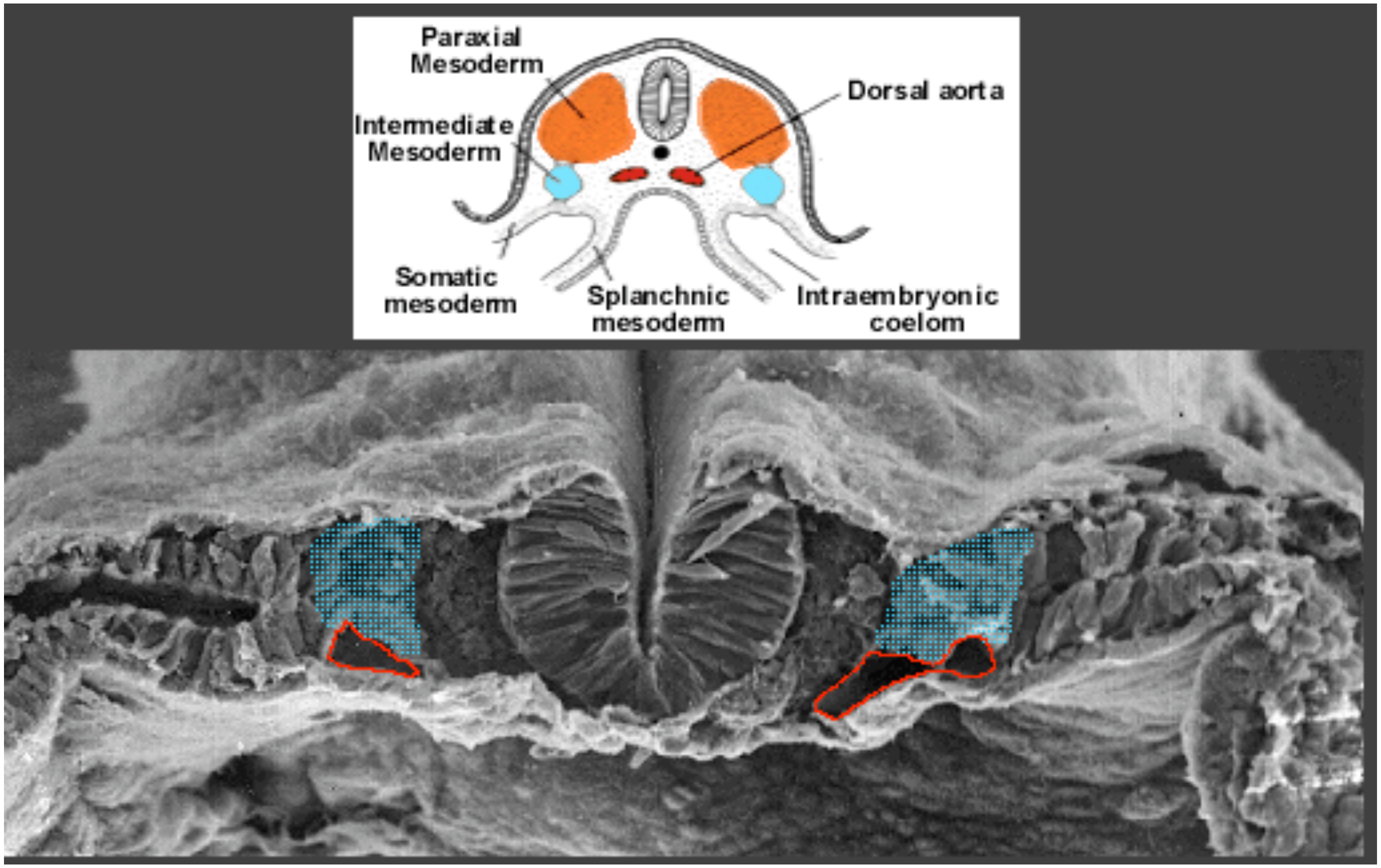
The papilla contains bundles of nephrons and CDs



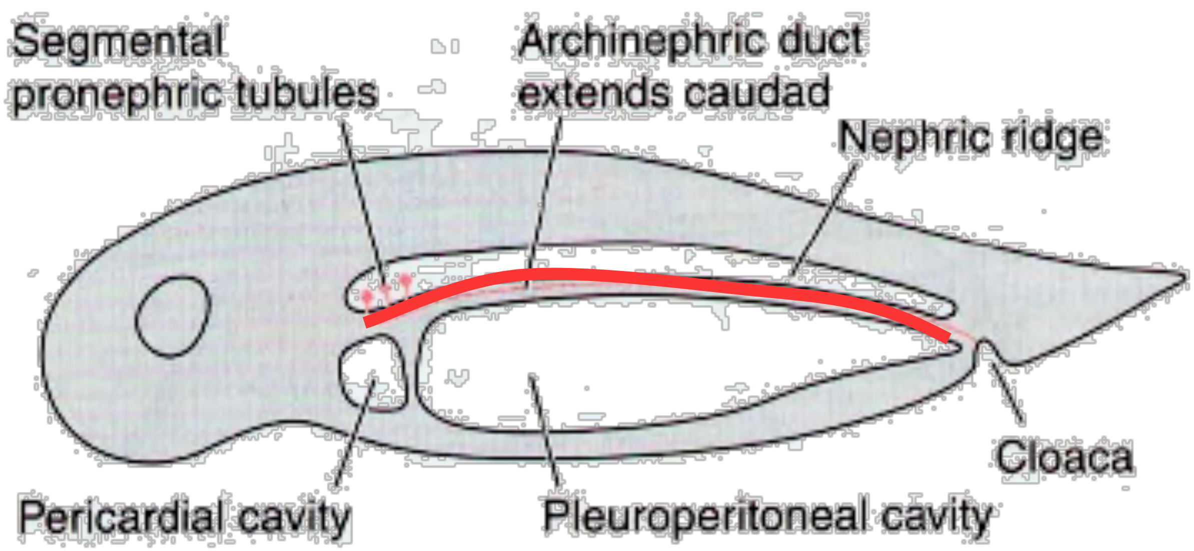




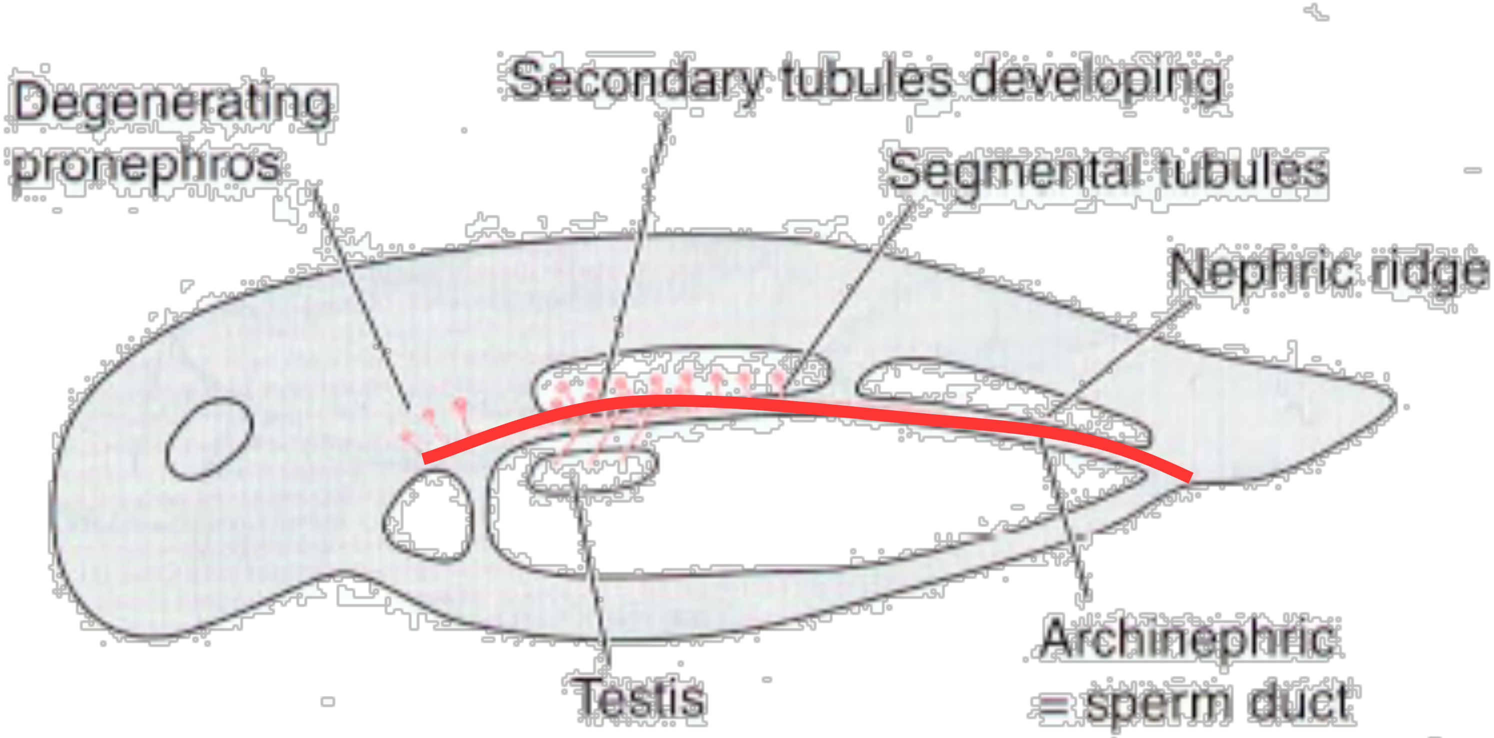
The urogenital system derives predominantly from intermediate mesoderm



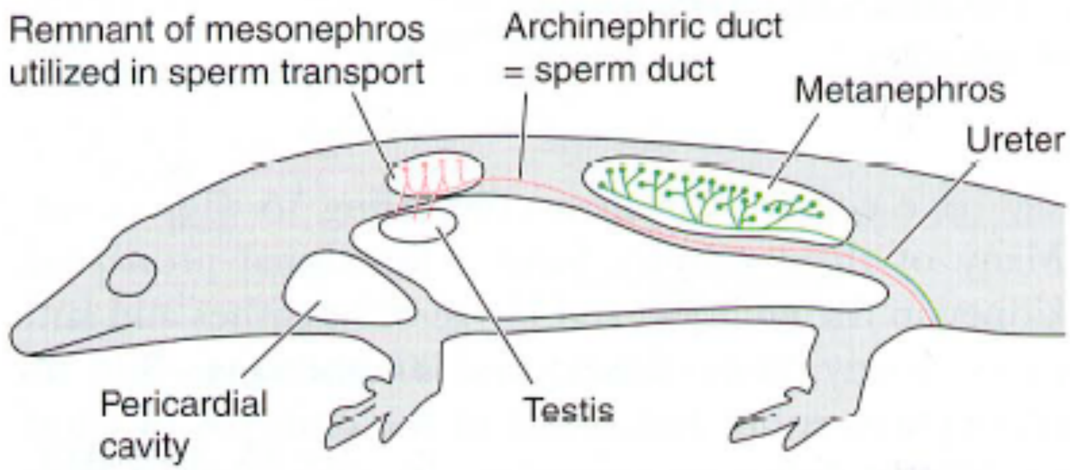
pronephros in an early embryo



The pronephros is a functional kidney in some species
but regresses in mammals



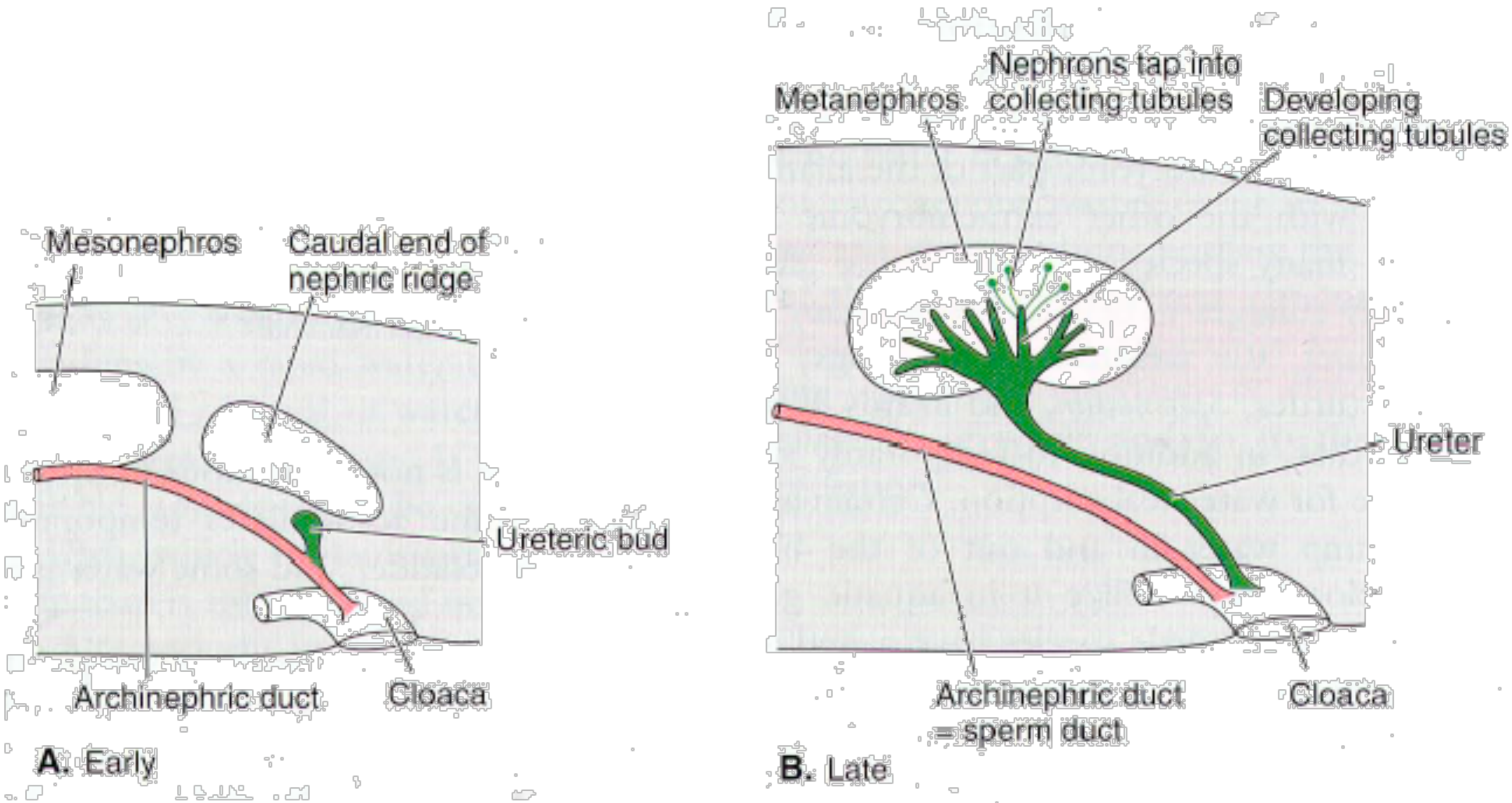
Mesonephros is functional in frogs and birds



C. Metanephros in late embryo and adult

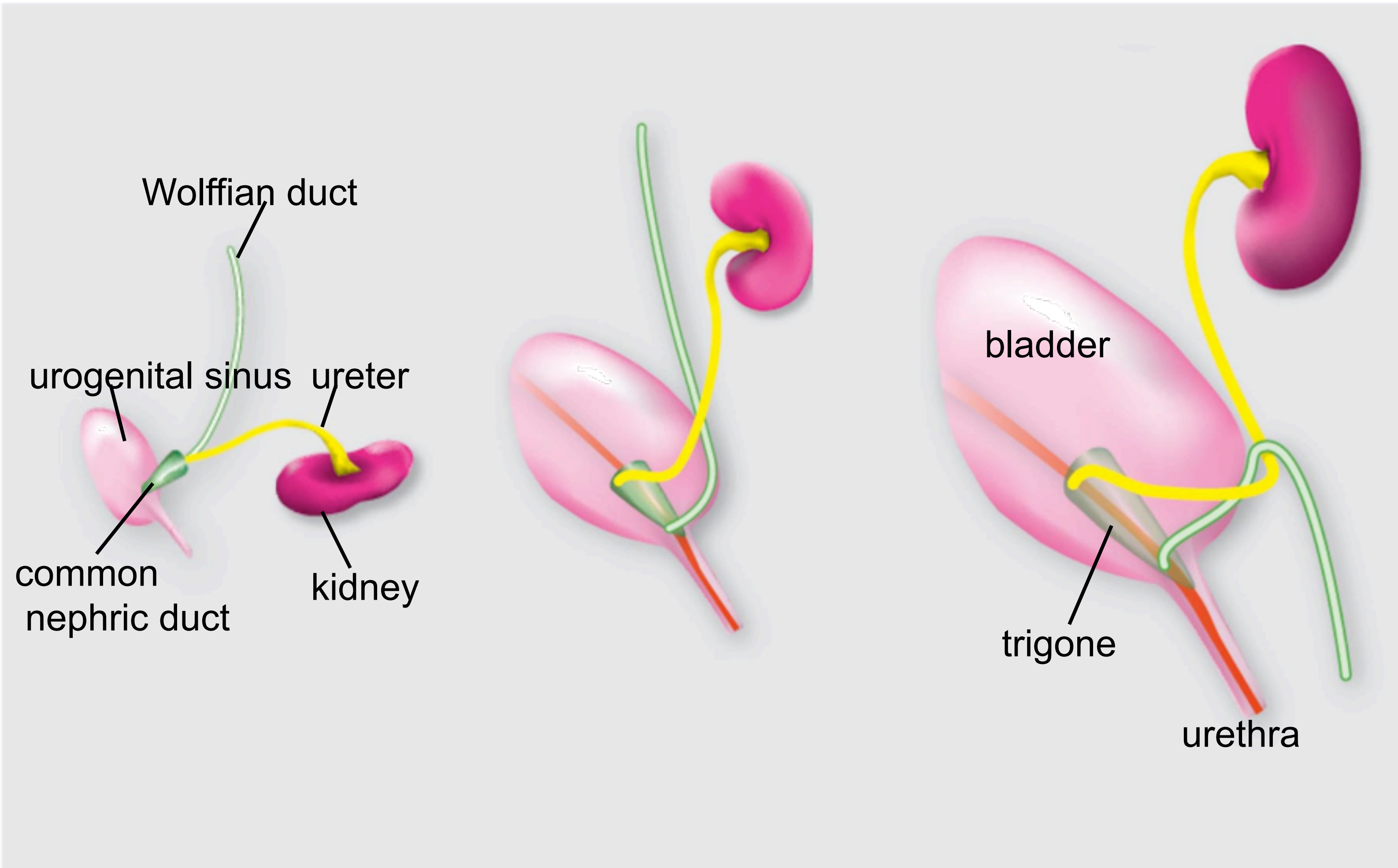
A **metanephros** is always drained exclusively by one duct, the ureter.

In birds in reptiles the ureter separates from the nephric duct (Wolffian duct) and enters the cloaca. In mammals, the ureter separates from the nephric duct and enters the bladder

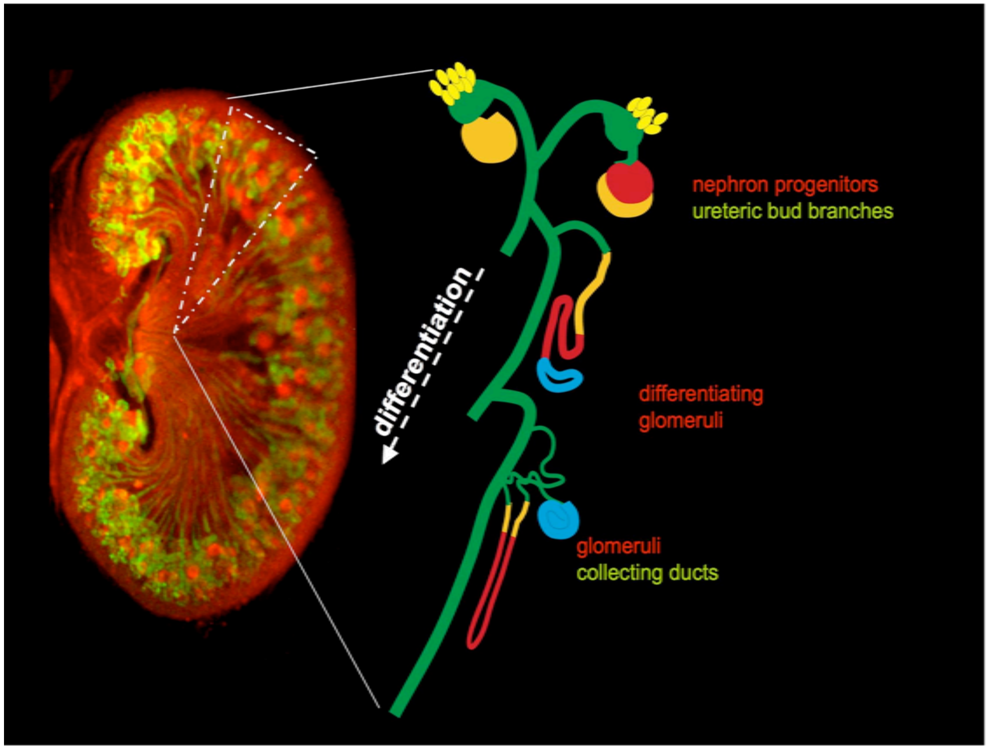


renal development begins when the **ureteric bud** invades kidney mesenchyme (**the metanephric blastema**)

As the embryo grows, the **ureters lengthen**, and the **kidneys rotate** and **ascend** along the dorsal body wall

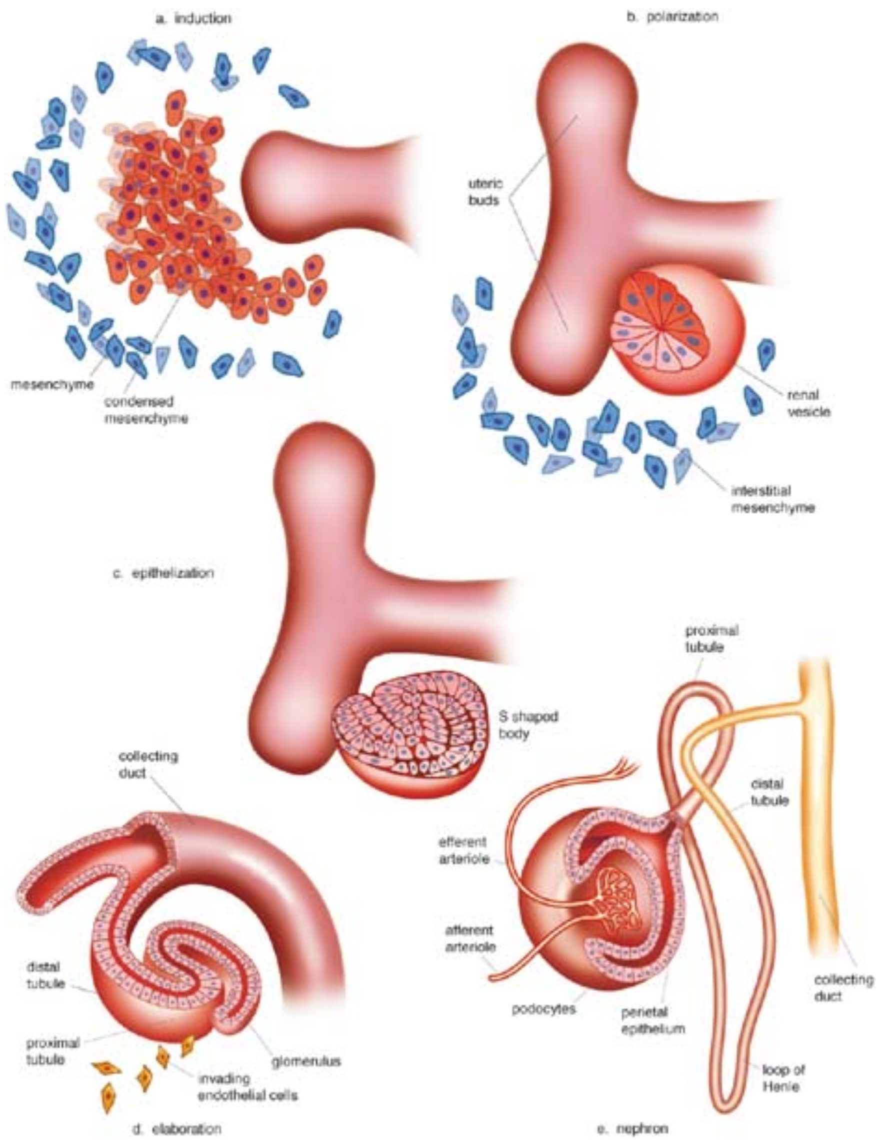


The kidney is radially patterned



- branching morphogenesis and nephron formation last until just after birth
- occur exclusively in the peripheral domain beneath the renal capsule
- new generations of nephrons and ureter branches displace older generations inward
- further differentiation occurs in inner domains at a distance from the renal capsule

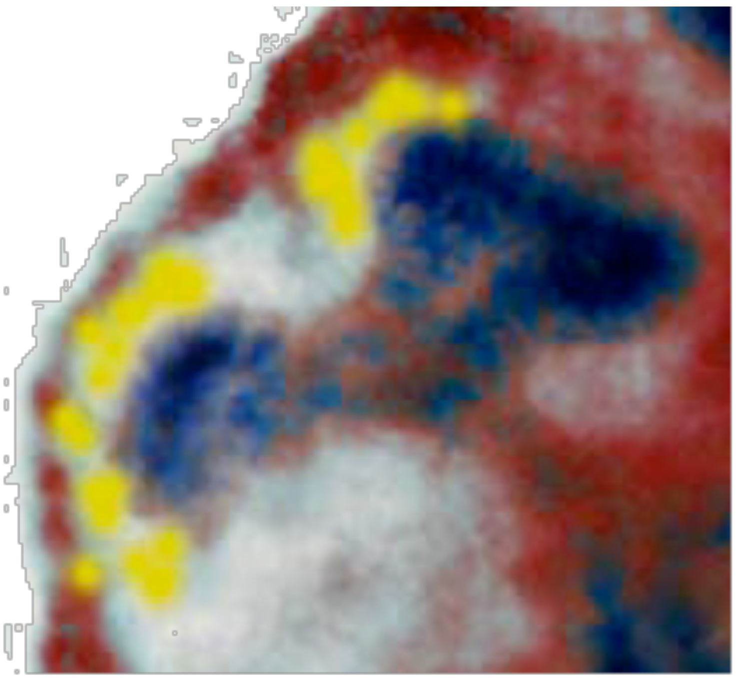
Nephron formation



From "The Kidney

Mesenchymal nephron progenitors aggregate at ub tips and transdifferentiate into epithelial cell types that comprise the nephron

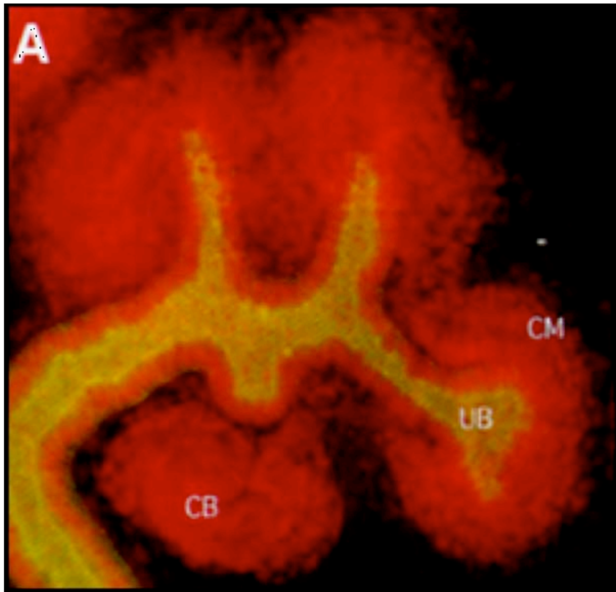
RECIPROCAL SIGNALING BETWEEN STROMA, NEPHRON PROGENITORS AND URETERIC BUD TIPS GIVES RISE TO CELL TYPES IN THE MATURE KIDNEY



nephron progenitors NEPHRONS

ureteric bud tips COLLECTING DUCT SYSTEM

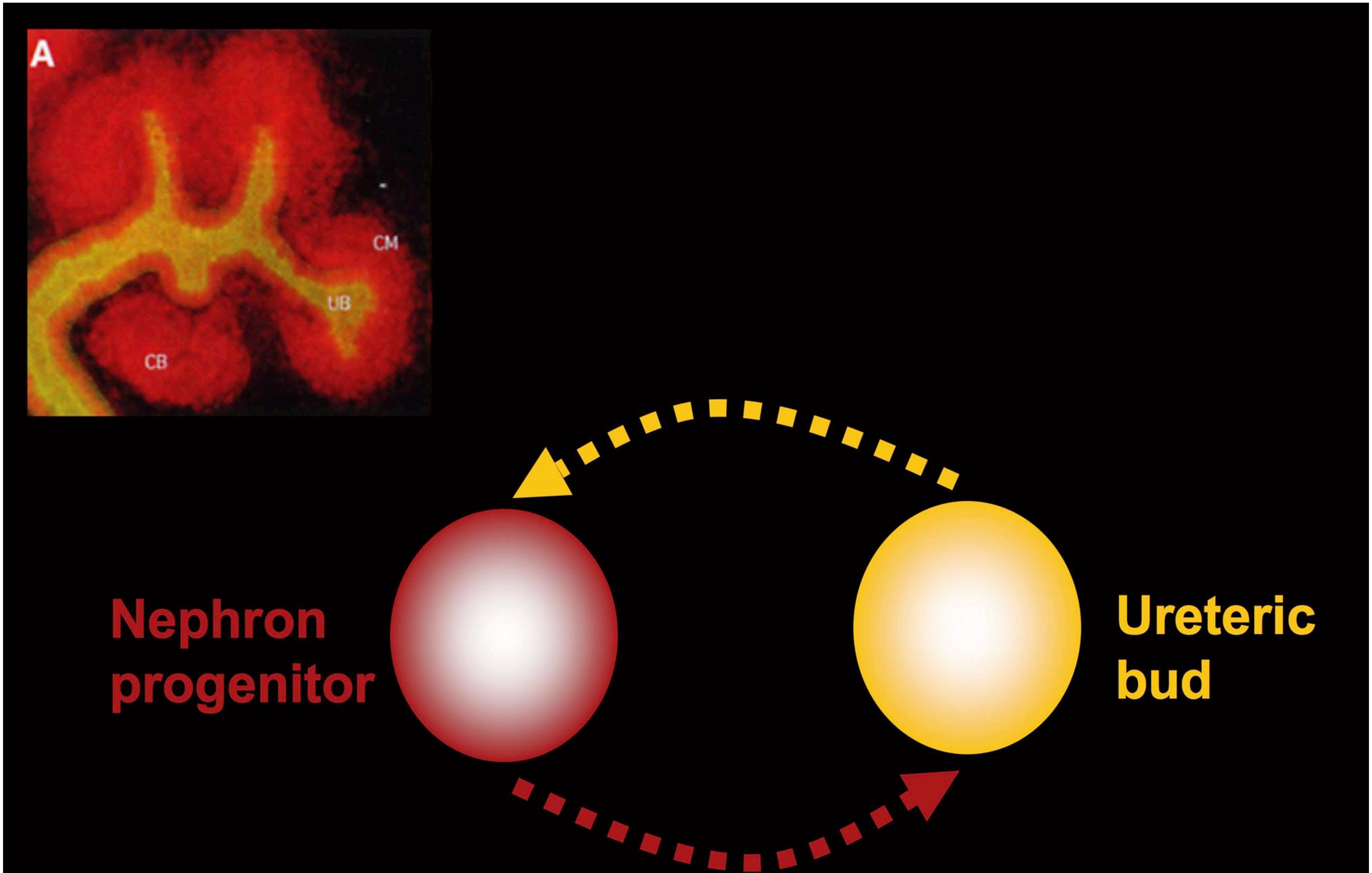
stroma CAPSULE/INTERSITIUM



Melissa Little Lab

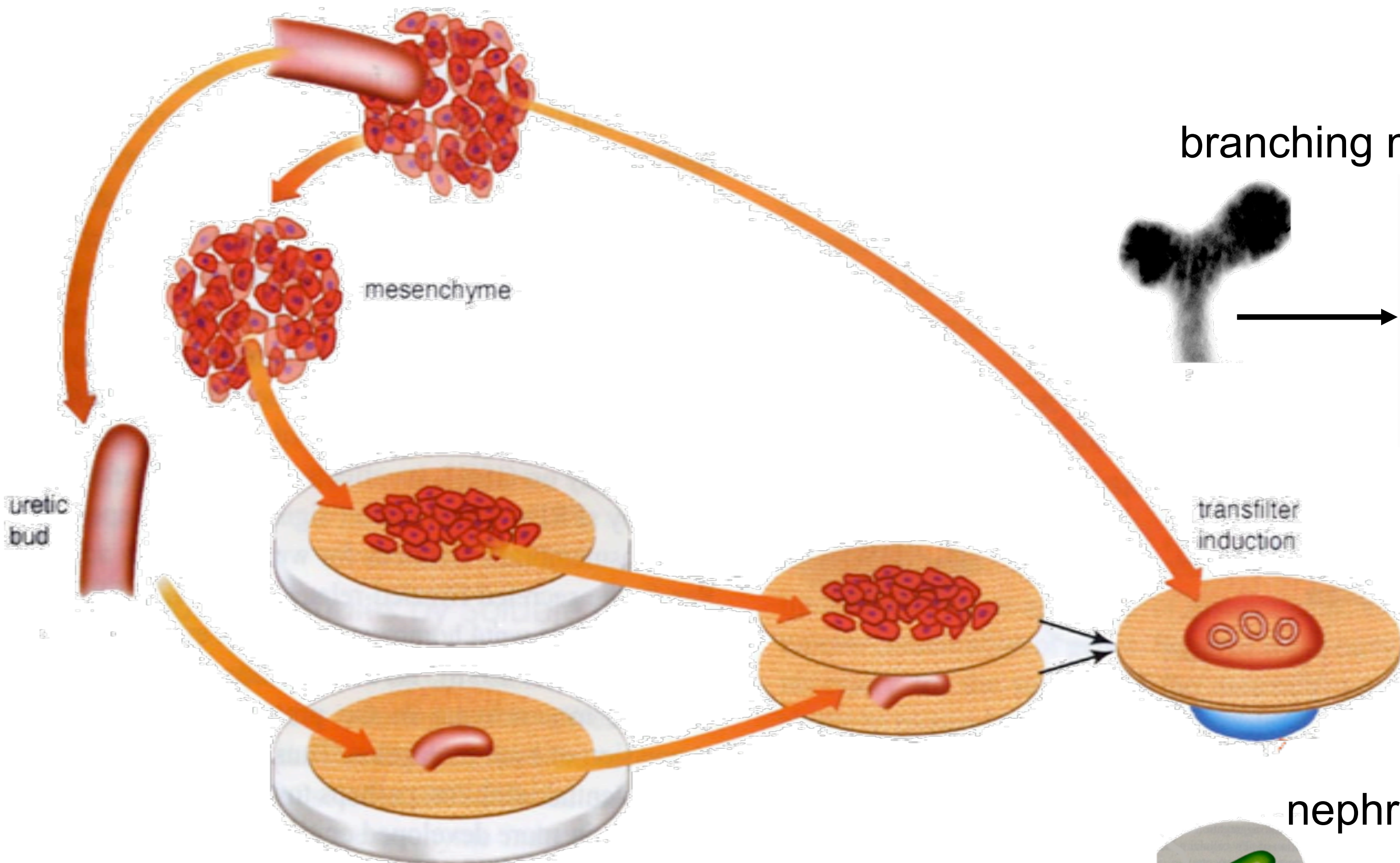
Nephron progenitors form nephrons

The ureteric bud forms the CD system

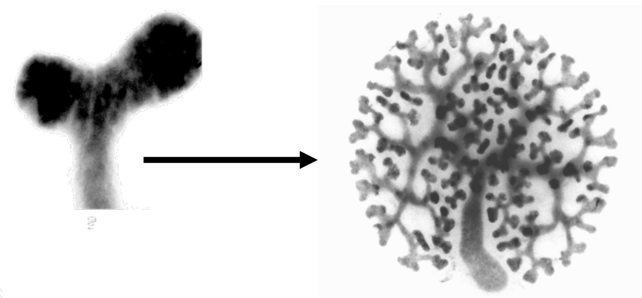


Reciprocal Signaling is required for branching morphogenesis and for nephron differentiation

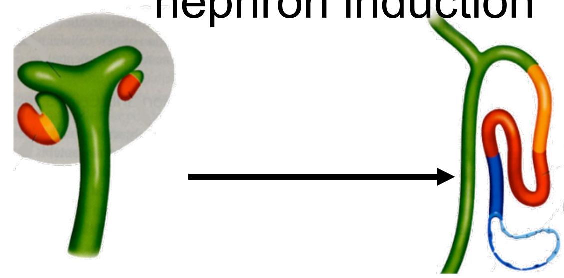
co-culture experiments demonstrate reciprocal signaling between ureteric bud epithelial and nephron progenitors



branching morphogenesis

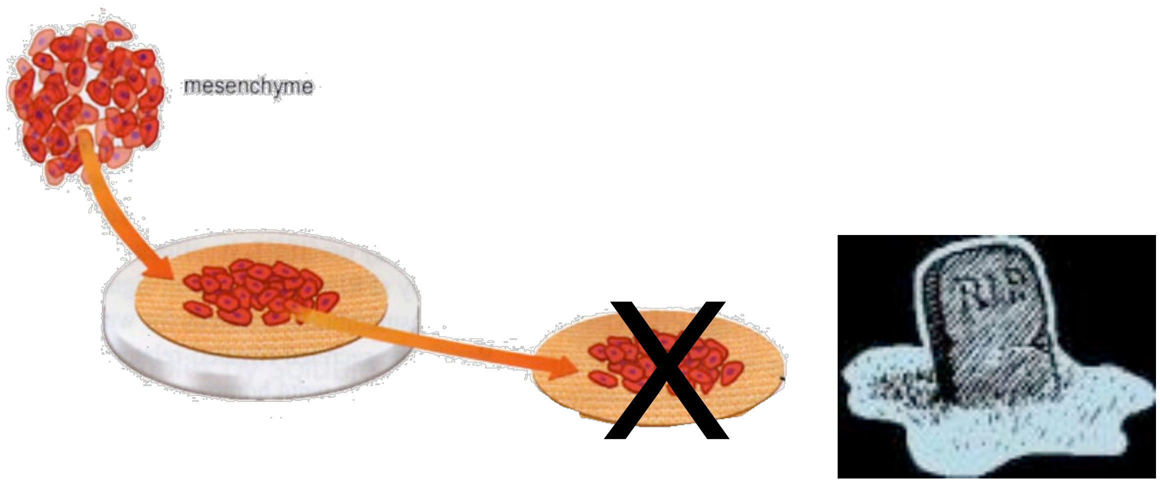


nephron induction



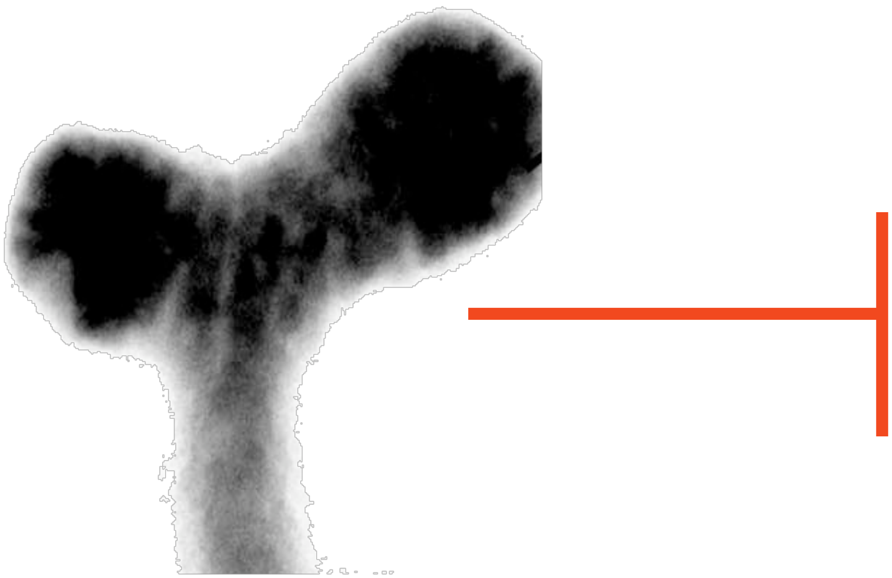
from: The kidney: Eds, Vize et al., 2003)

• no ureteric bud, nephron progenitors undergo apoptosis



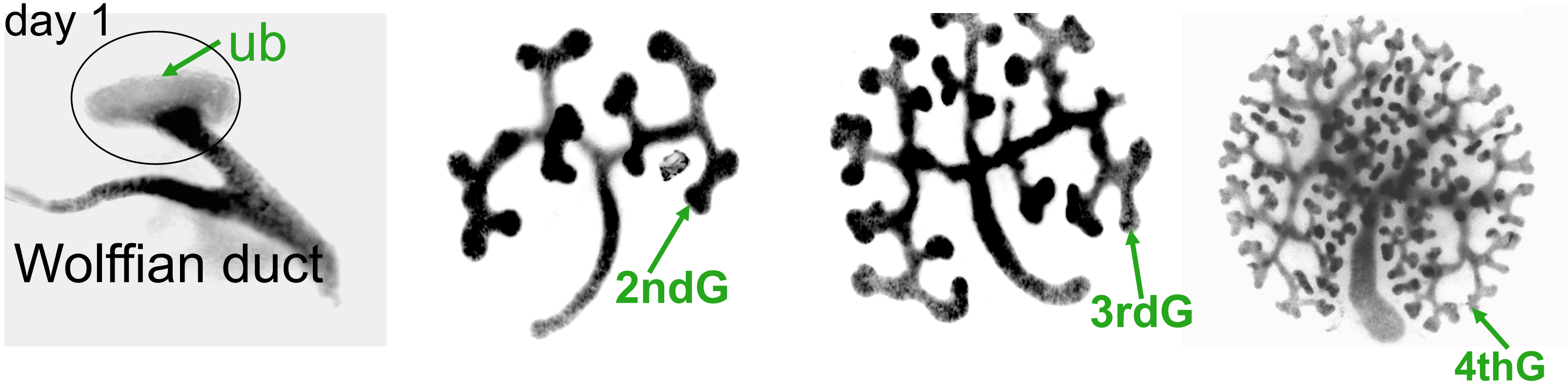
from: The kidney: Eds, Vize et al., 2003)

•no nephron progenitors, no branching morphogenesis



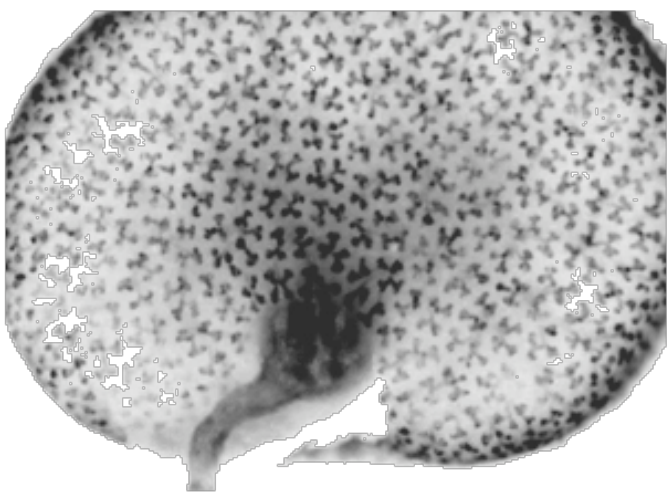
signals from the ureteric bud control nephron induction

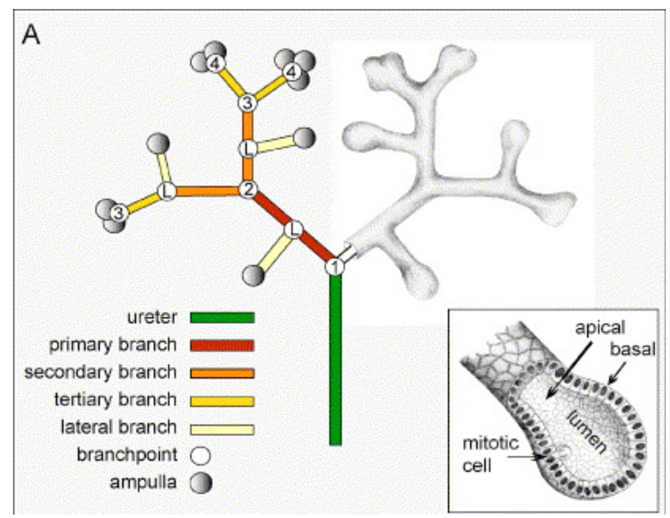
signals from nephron progenitors control branching morphogenesis



The collecting duct system grows from the periphery by **dichotomous branching**

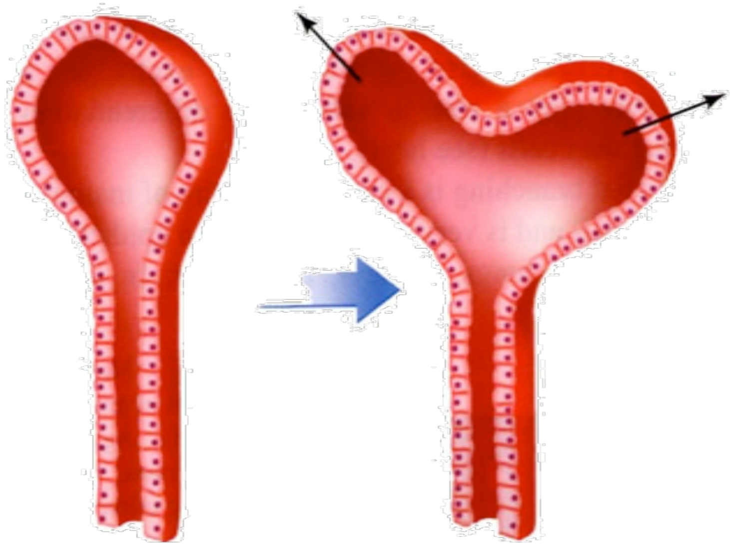
at birth:

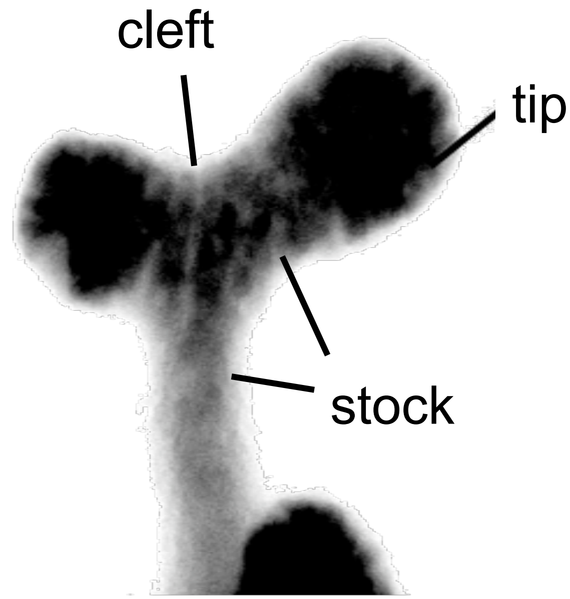




Hoxb7-Gfp expression showing the mouse collecting duct system

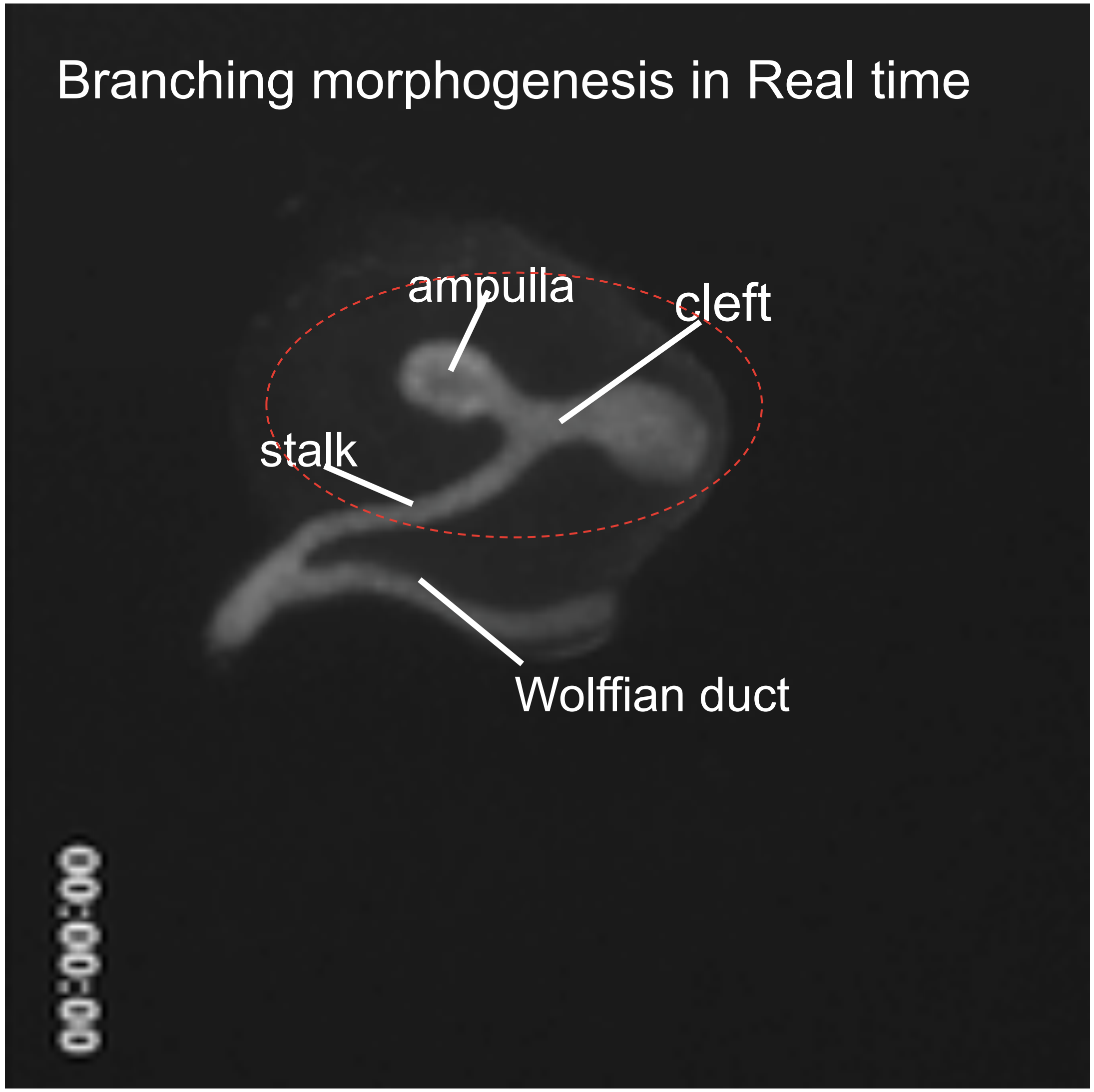
shape changes, local proliferation and remodeling at ureteric bud tips forms an ampulla





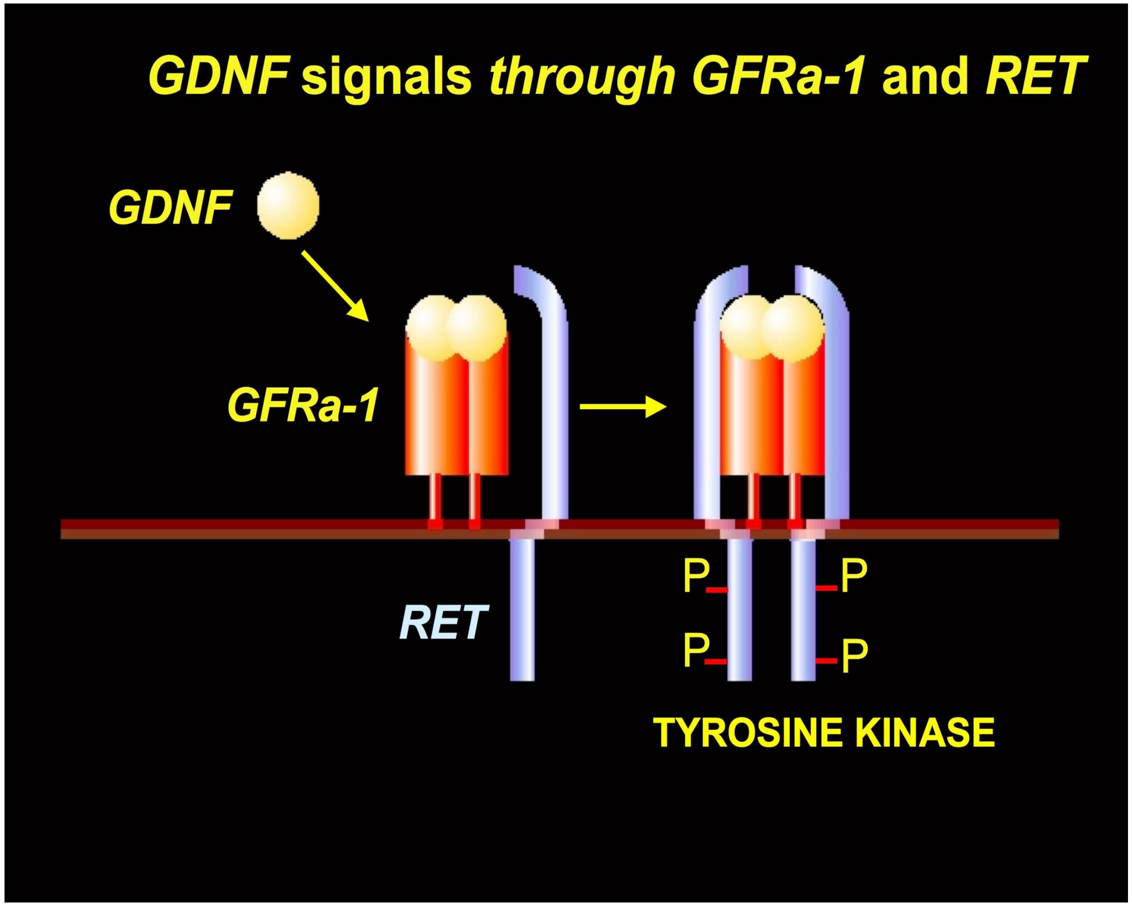
Branching morphogenesis:

- ampullae form at ureteric bud tips
- a cleft forms and the tips begin to bifurcate
- the tips elongate
- new ampullae form

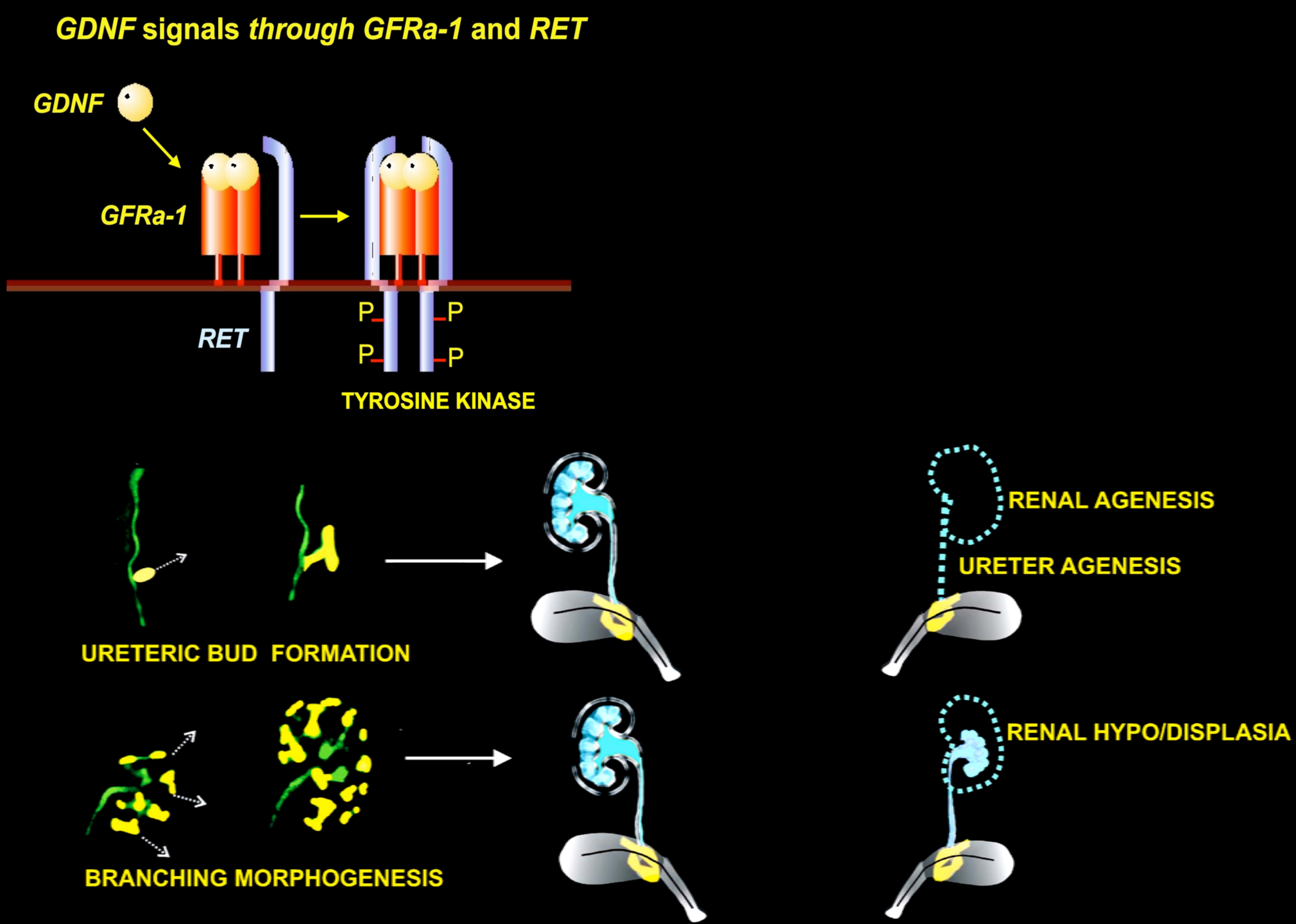


Costantini Lab
Columbia University, Dept. of Genetics & Development

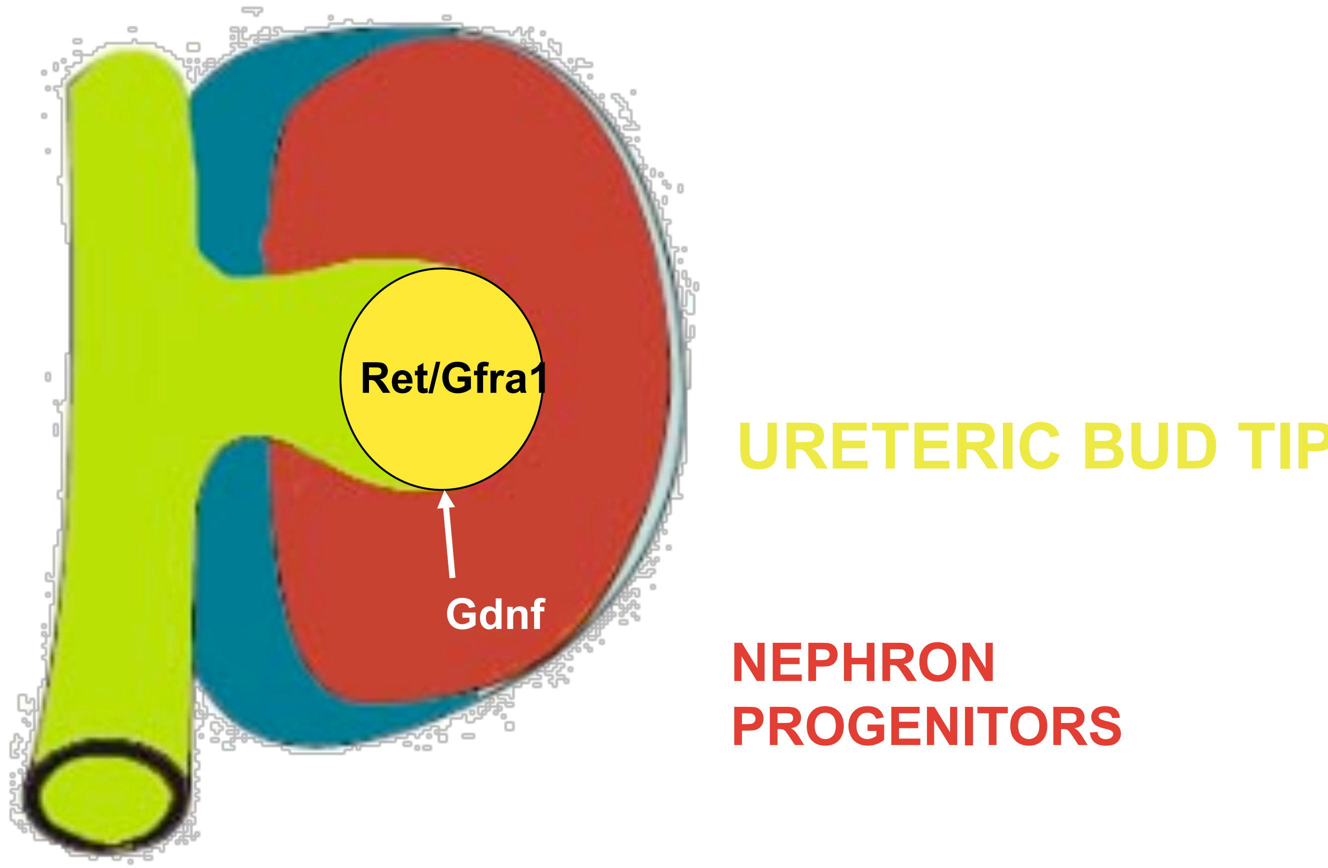
RET-Gdnf signaling is crucial for branching morphogenesis



Ret mutations in humans cause renal abnormalities, Hirschsprung's disease and cancer

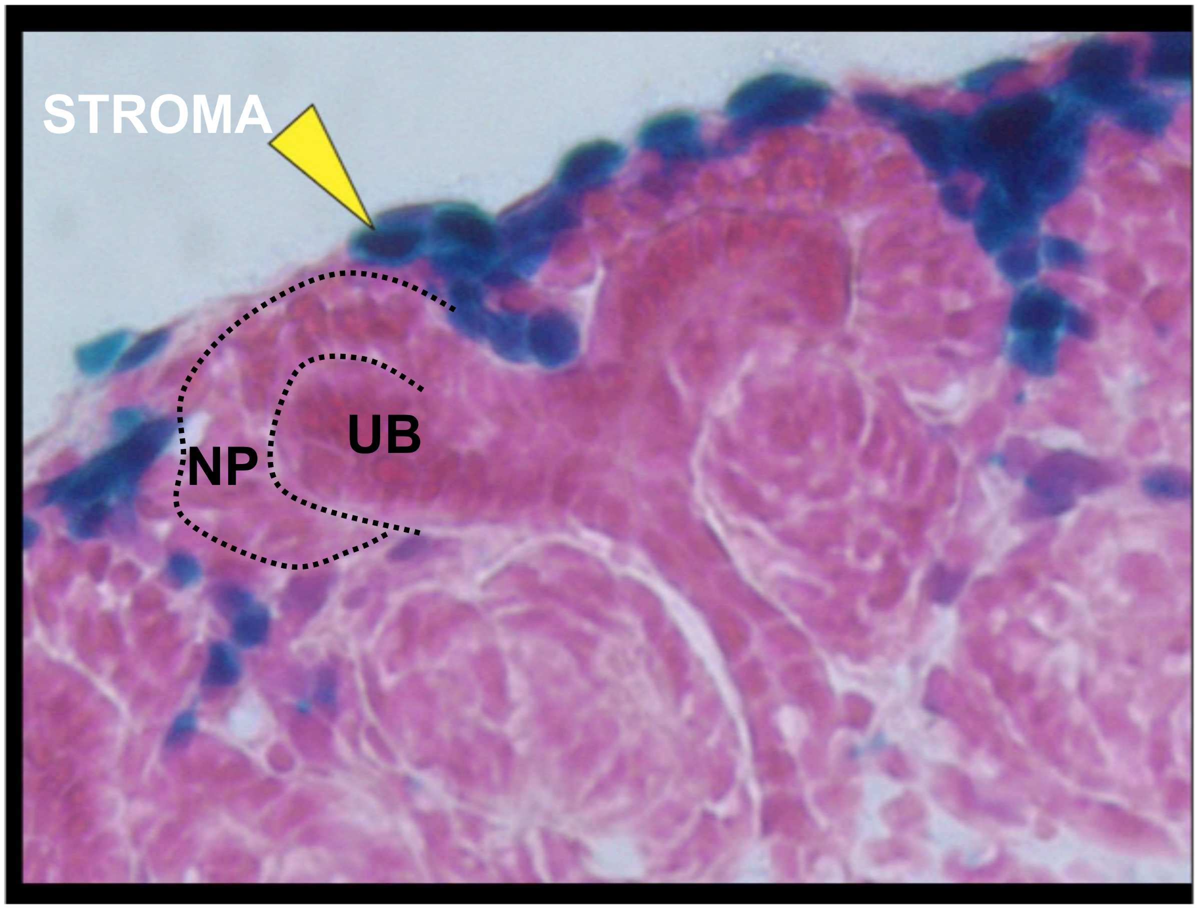


Mutations in Ret, Gdnf or Gfra1 result in renal agenesis or hypoplasia

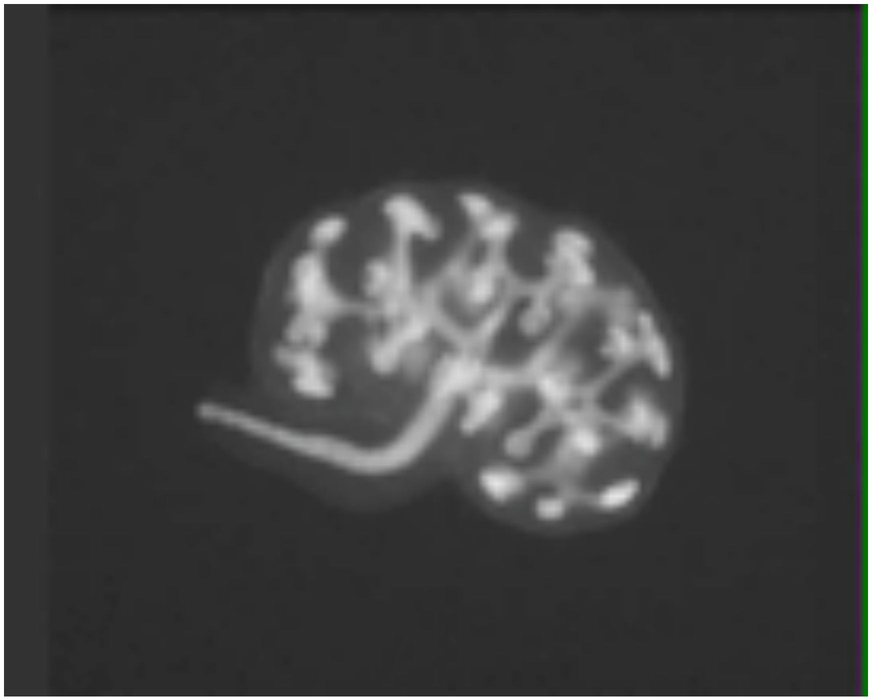


Gdnf secreted by nephron progenitors binds to Ret via the Ret co-receptor (Gfra1) inducing branching

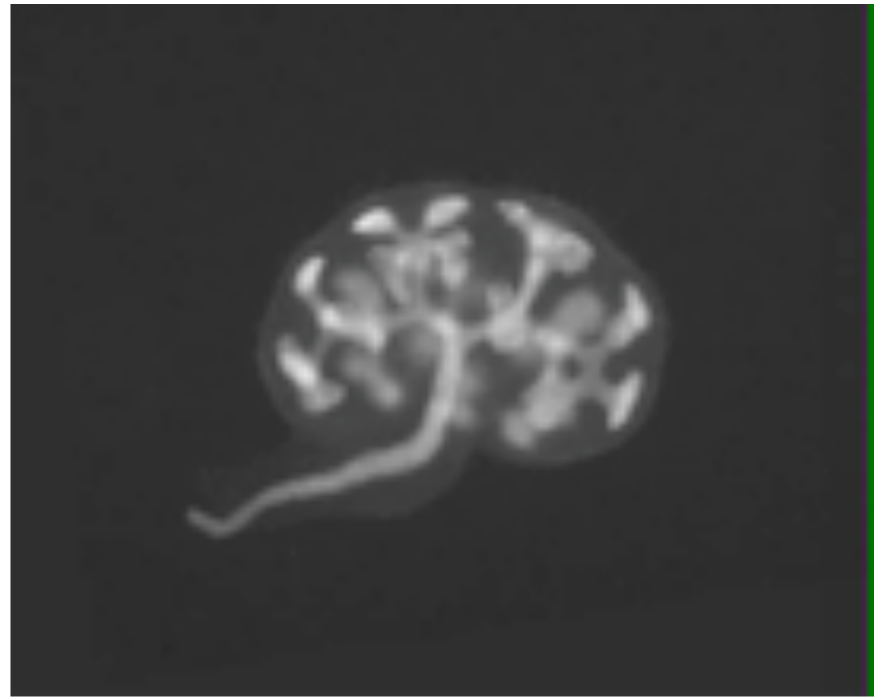
STROMAL CELLS SECRETE RETINOIC ACID THAT IS REQUIRED FOR BRANCHING AND RET EXPRESSION IN UB TIPS



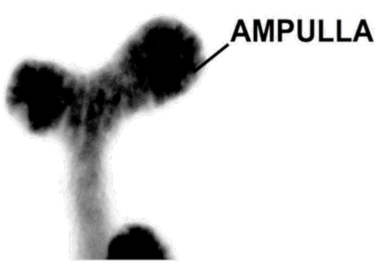
TIME-LAPSE PHOTOGRAPHY OF HOXB7-GFP KIDNEYS GROWN WITH AND WITHOUT RA



RA



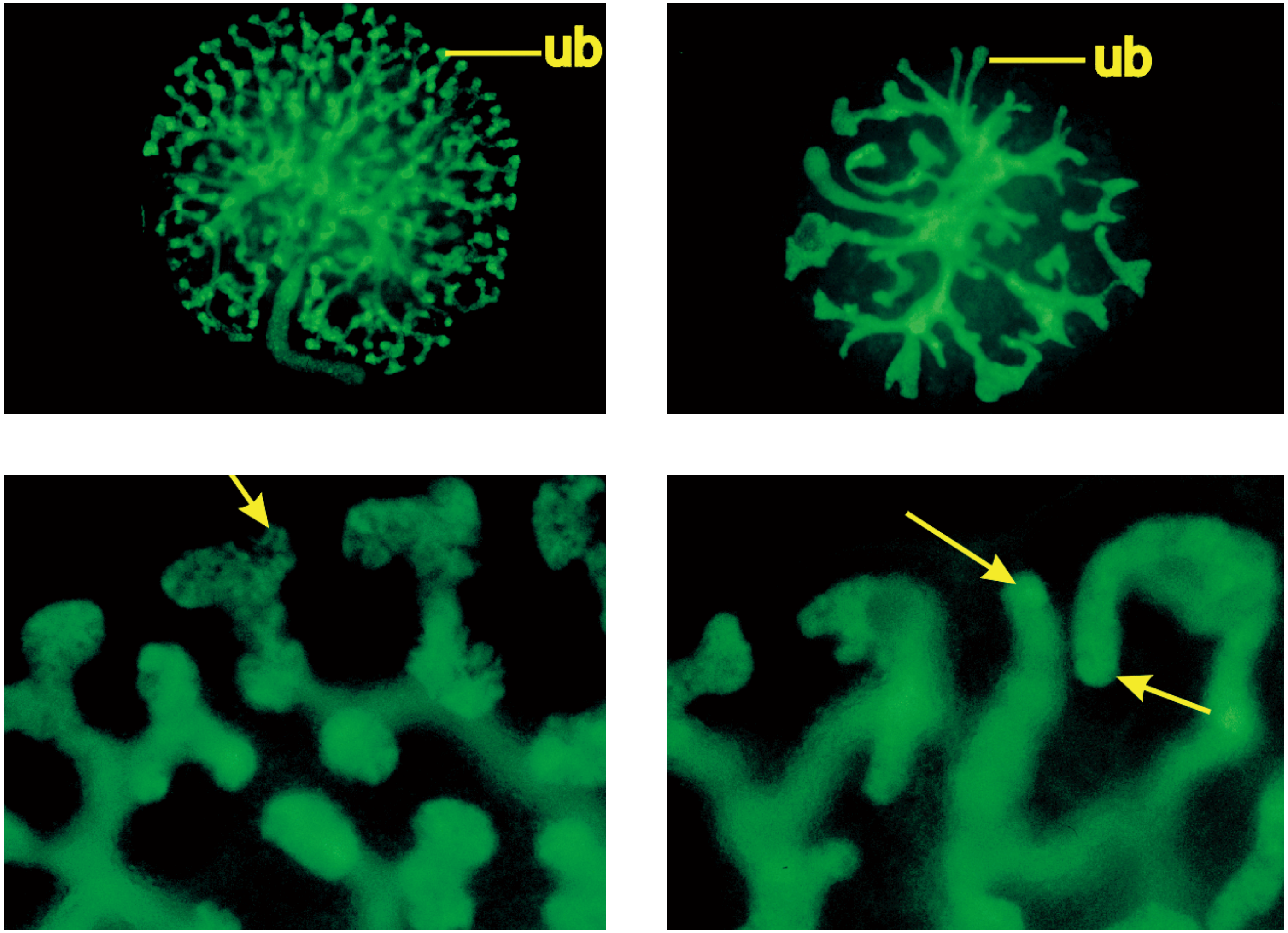
Control



AMPULLA FORMATION DEPENDS ON RA

Hoxb7-Gfp kidney rudiments

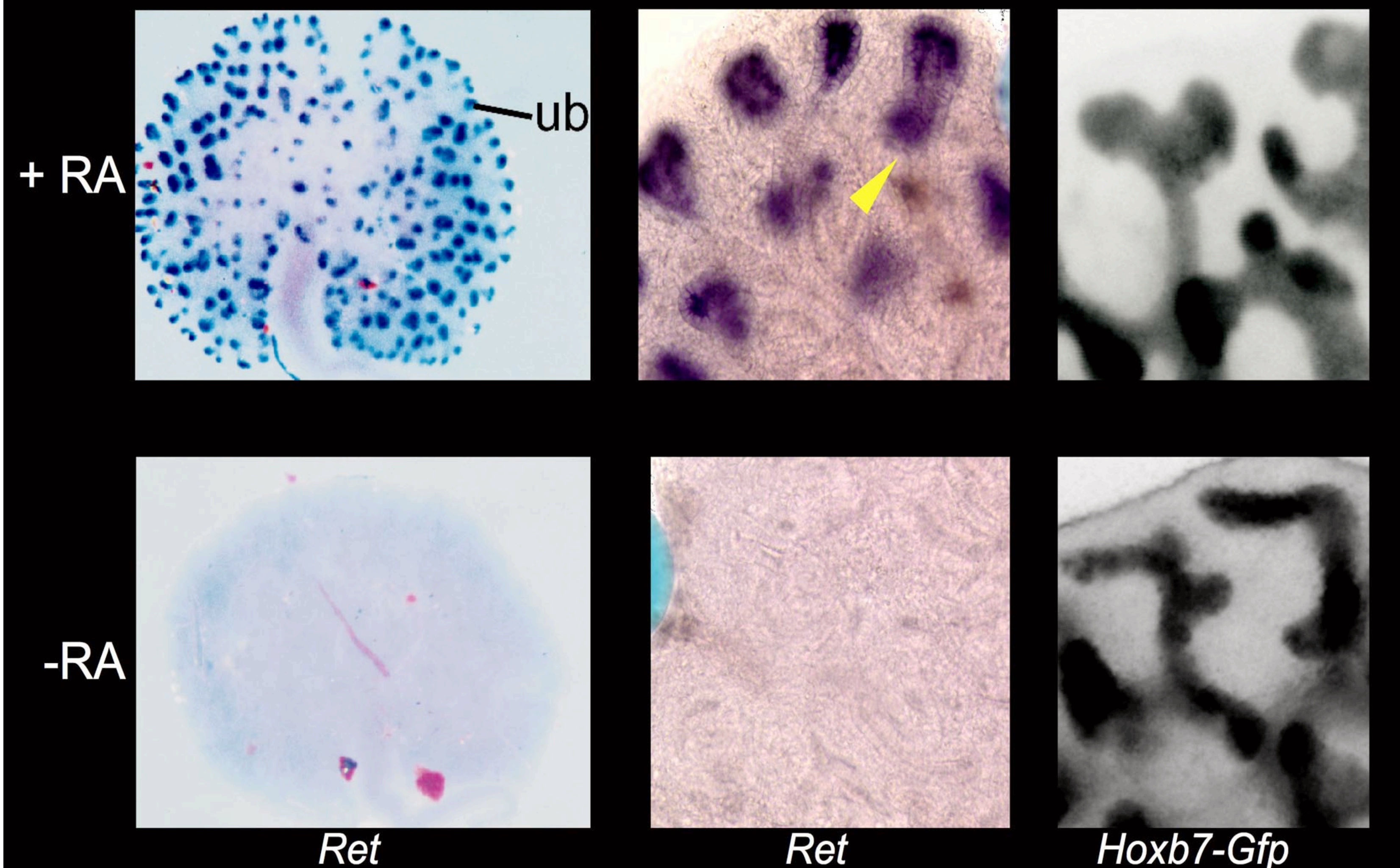
Retinoic acid controls ureteric bud patterning



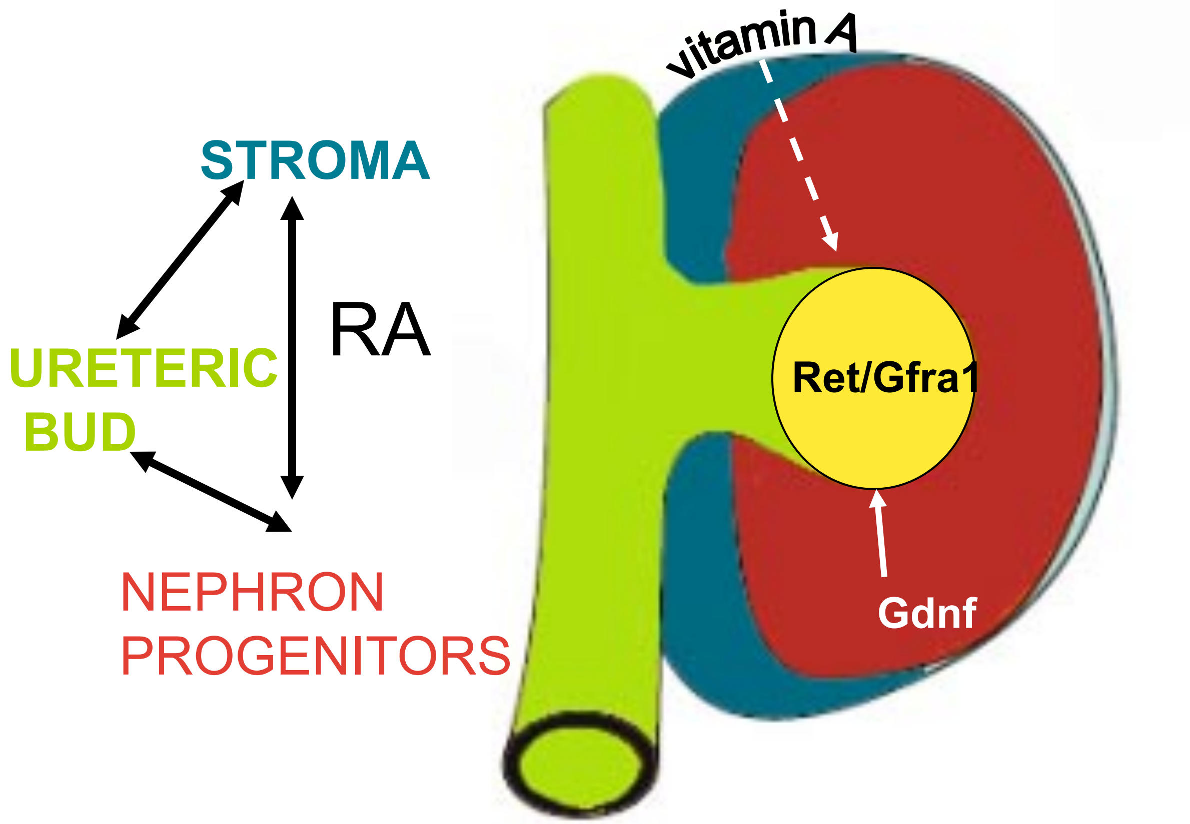
+Retinoic acid 72h

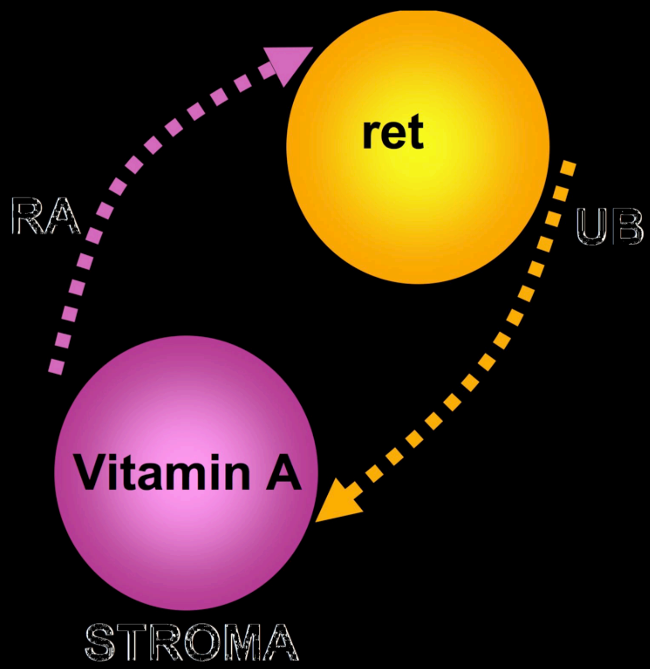
-Retinoic acid 72h

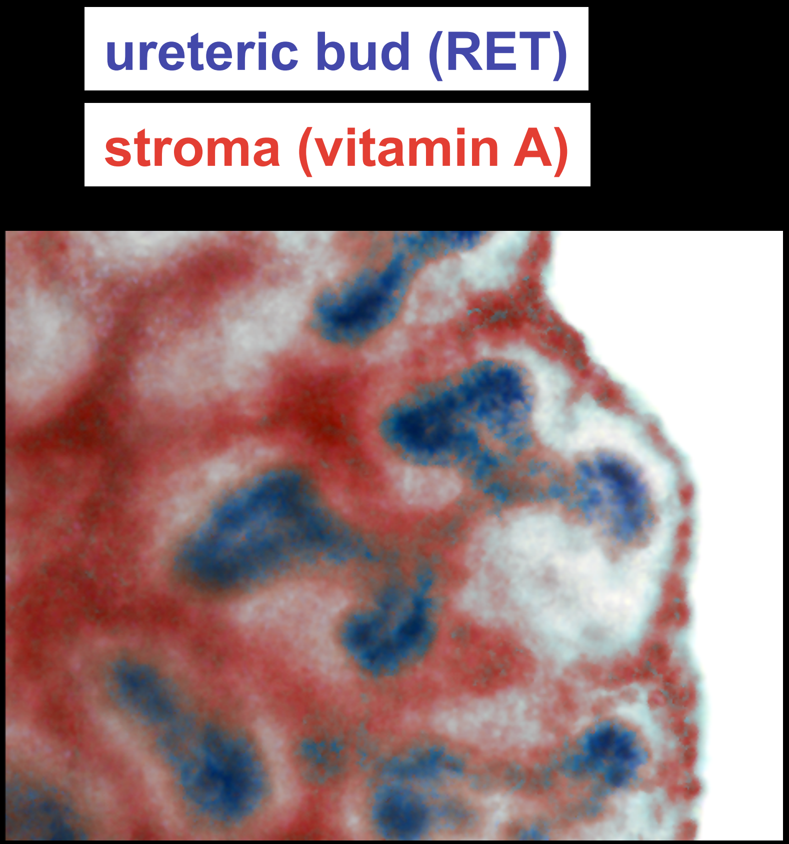
Ret expression in the ureteric bud depends on Retinoic acid



STROMAL CELL SECRETE RETINOIC ACID
RETINOIC ACID INDUCES RET EXPRESSION IN NEARBY URTERIC BUD CELLS



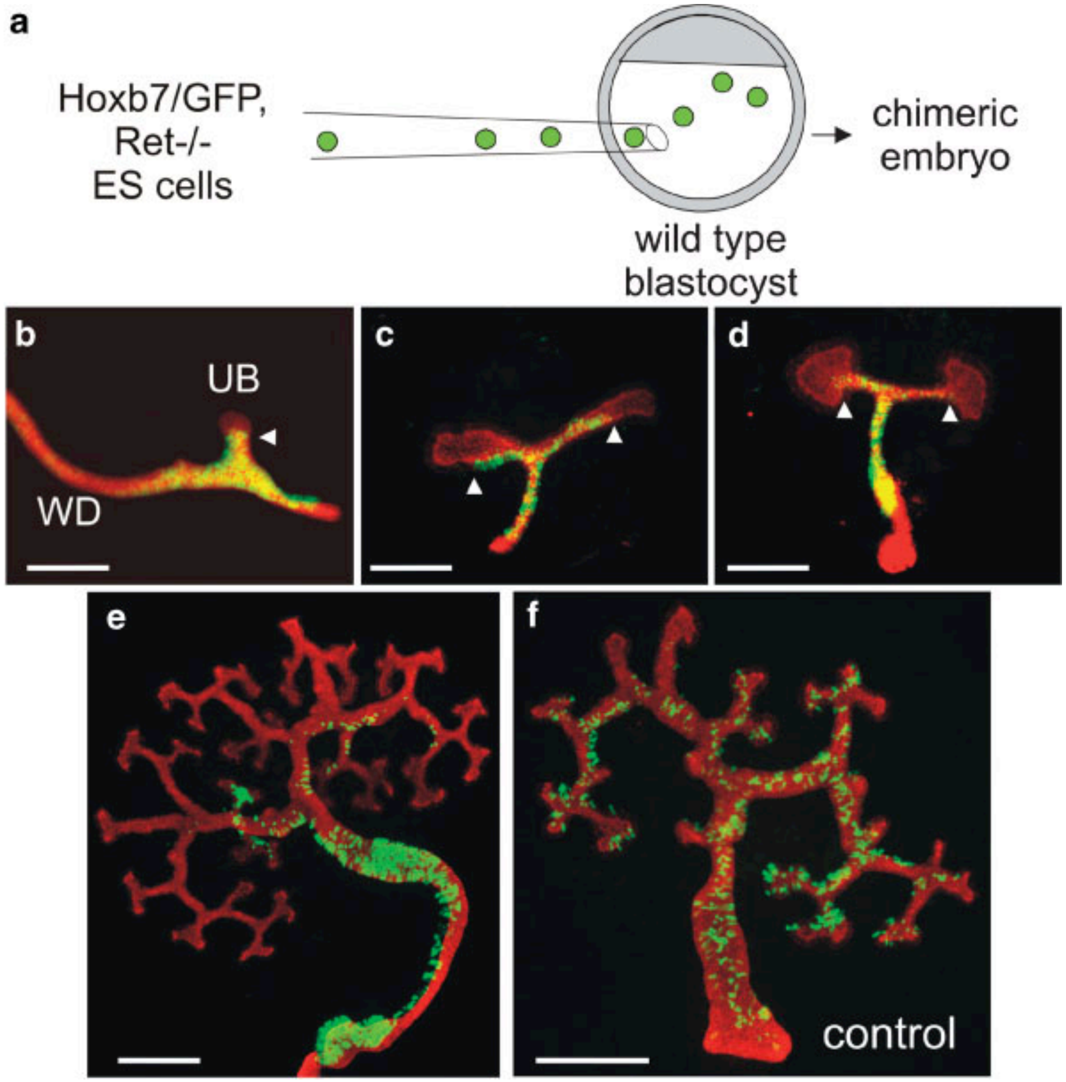




RA secreted from stromal cells controls Ret expression In the ureteric bud

How does Ret signaling control branching?

ureteric bud cells must express Ret to contribute to a tip

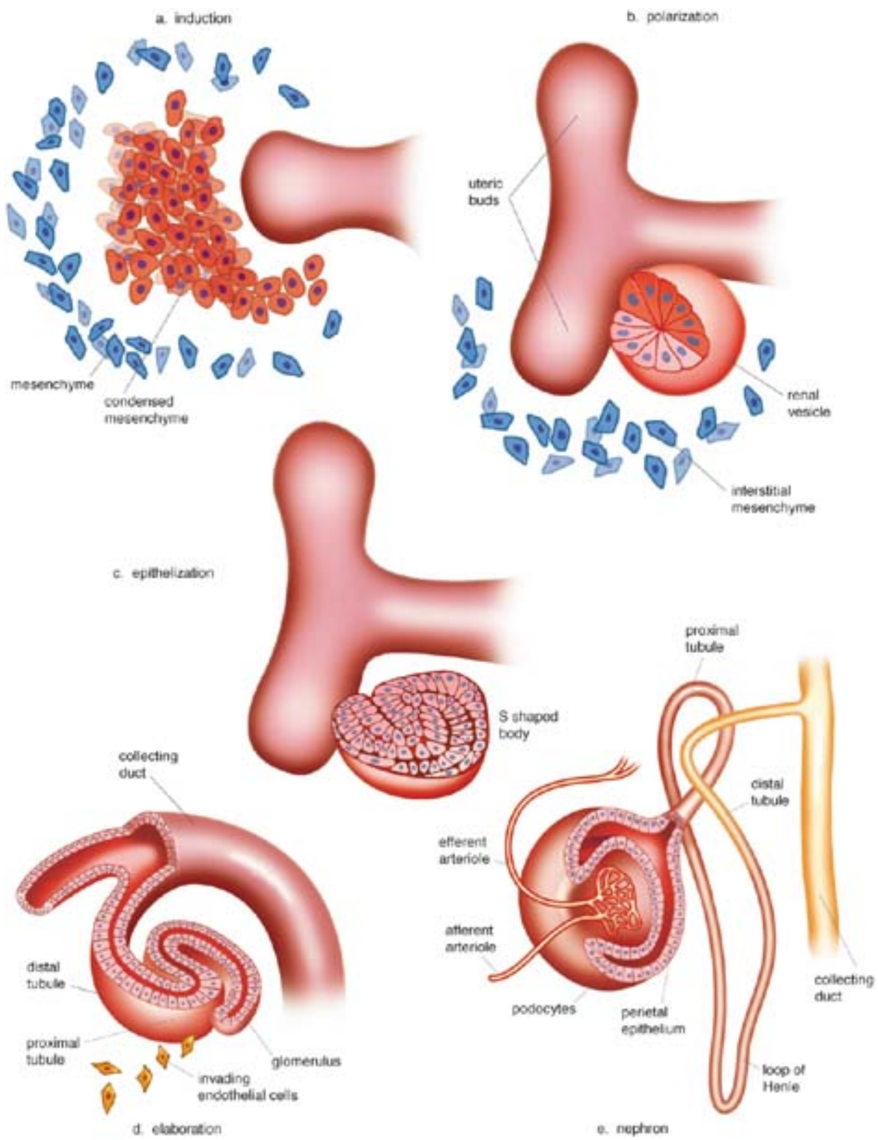


Ret null cells are excluded from the tip domain-

Presence in the tip depends on levels of Ret-Gdnf signaling?

How do nephrons form?

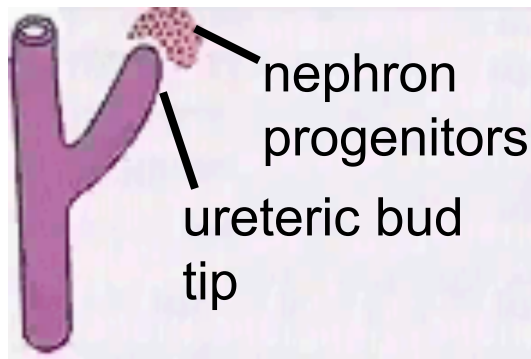
Nephron formation



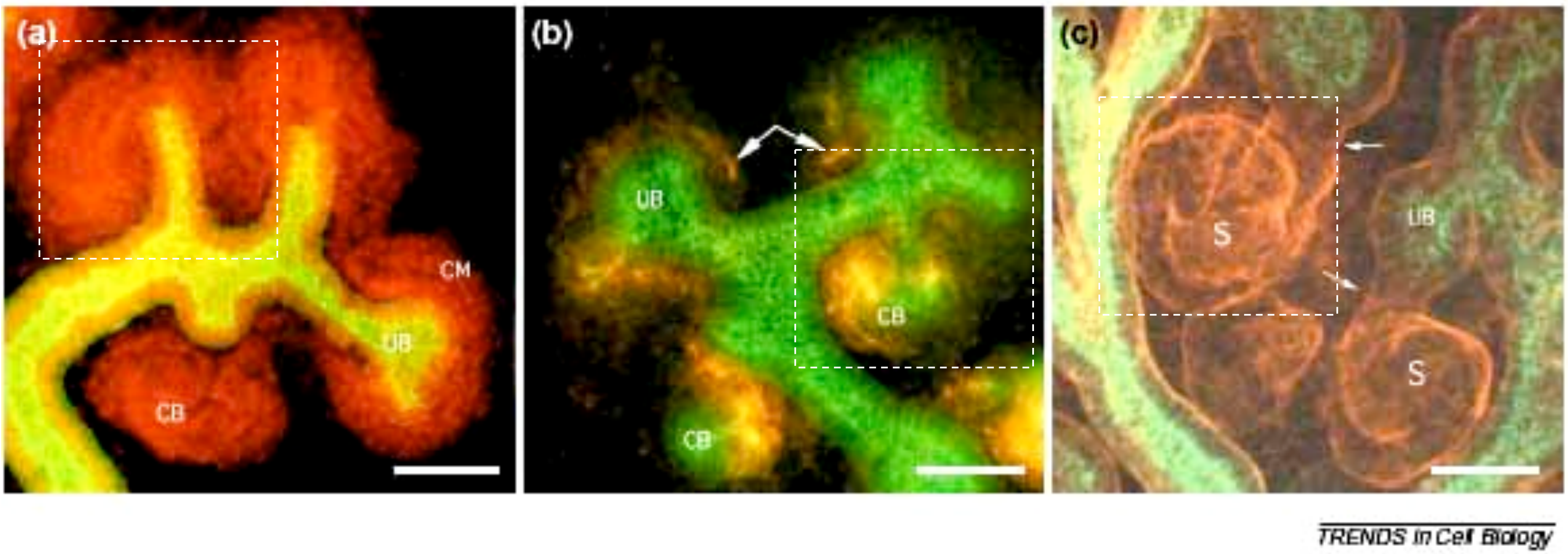
From “The Kidney

Mesenchymal nephron progenitors aggregate at ub tips and transdifferentiate into epithelial cell types that comprise the nephron

NEPHRONS FORM EXCLUSIVELY AT **URETERIC BUD TIPS** IN RESPONSE TO LOCAL SIGNALS FROM URETERIC BUD CELLS

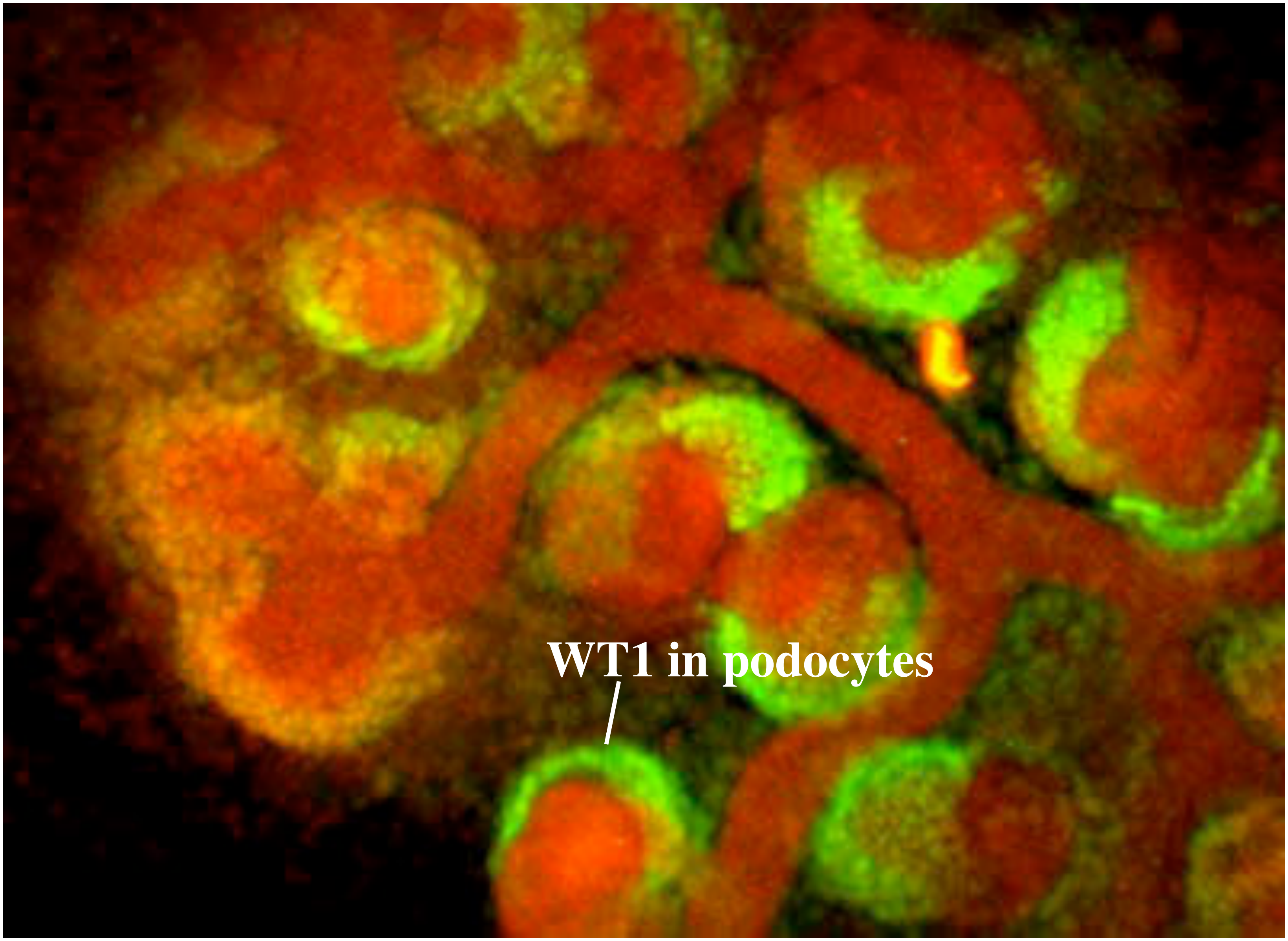


Nephron
progenitors condense at ub tips, aggregate



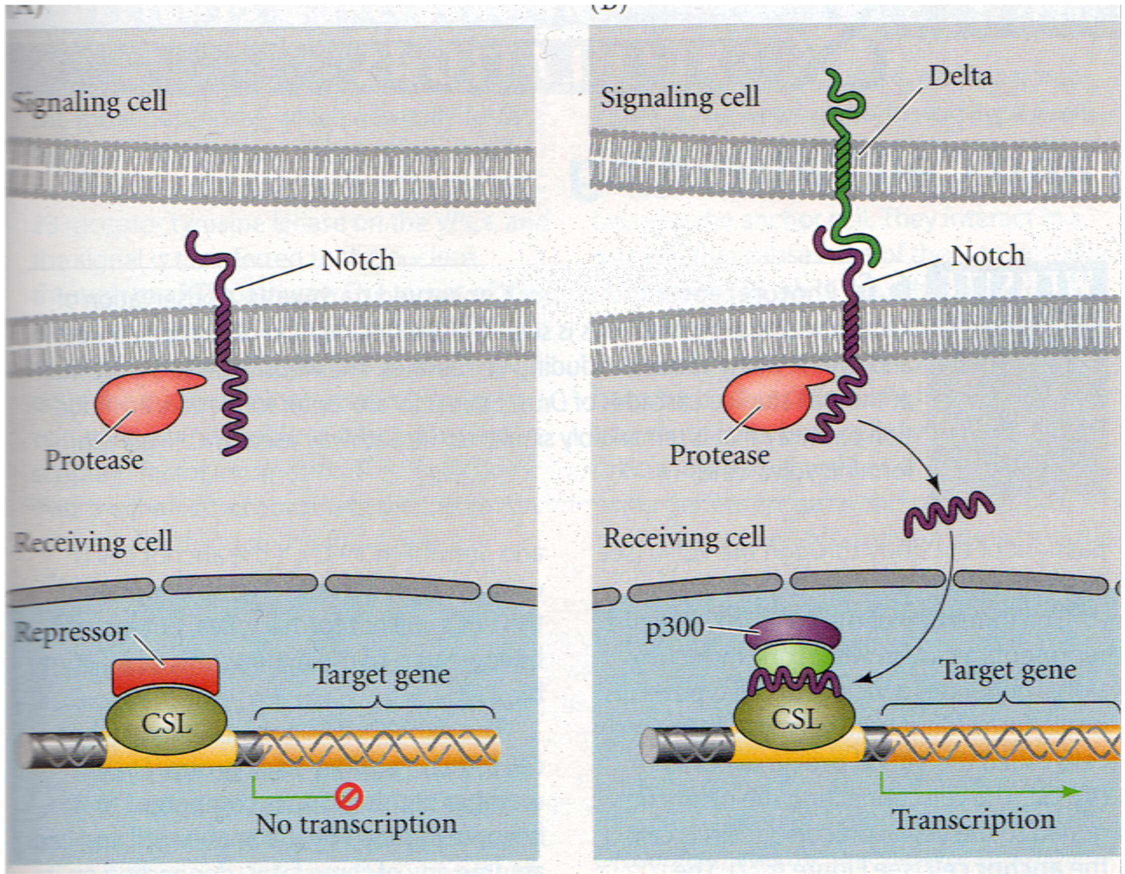
and trans-differentiate into epithelial cells
that make up the renal vesicle, Comma and S-shaped bodies

from: The kidney: Eds, Vize et al., 2003)

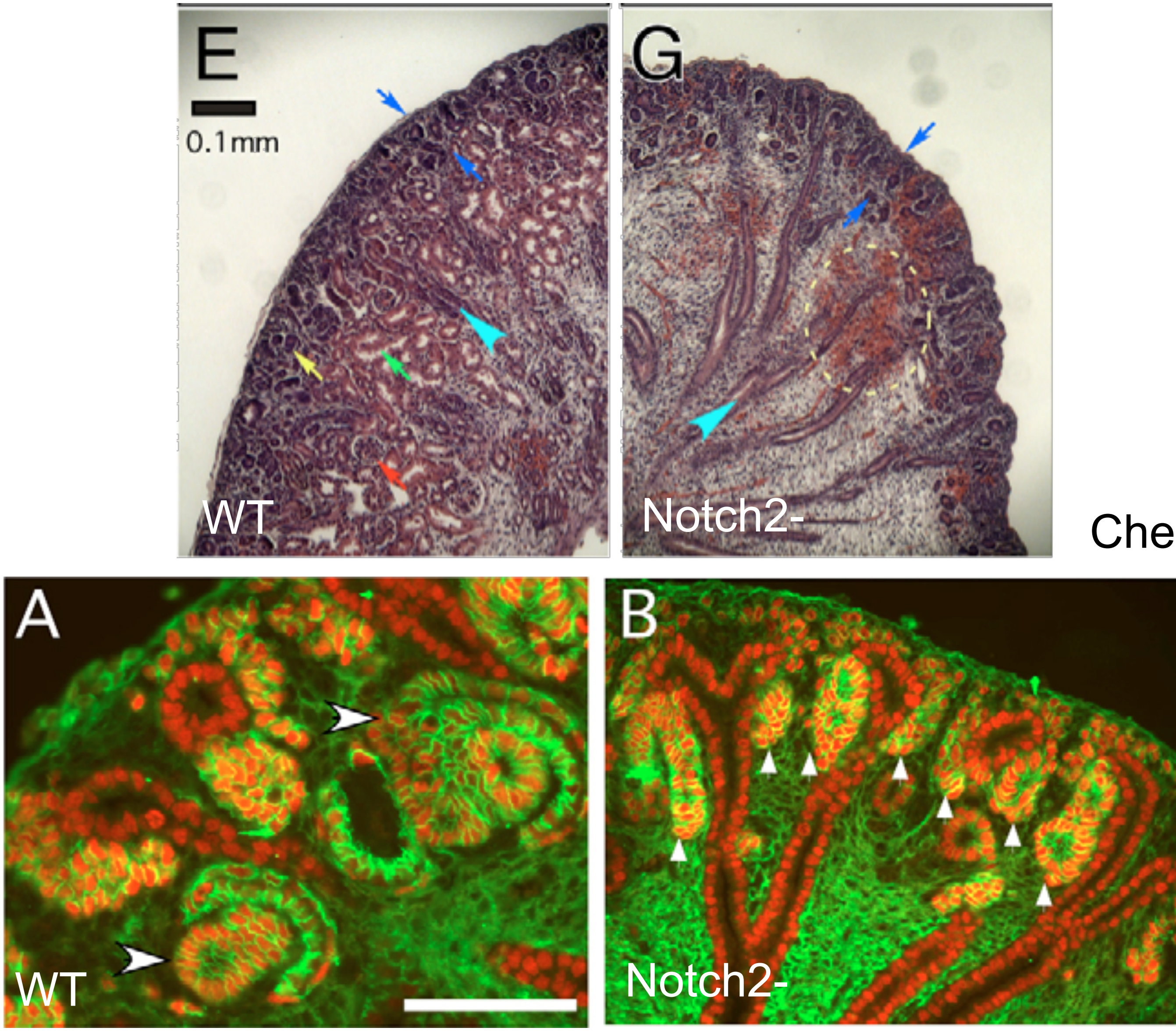


Nephron polarity is established at early stages

Notch signaling controls nephron patterning along the P-D axis



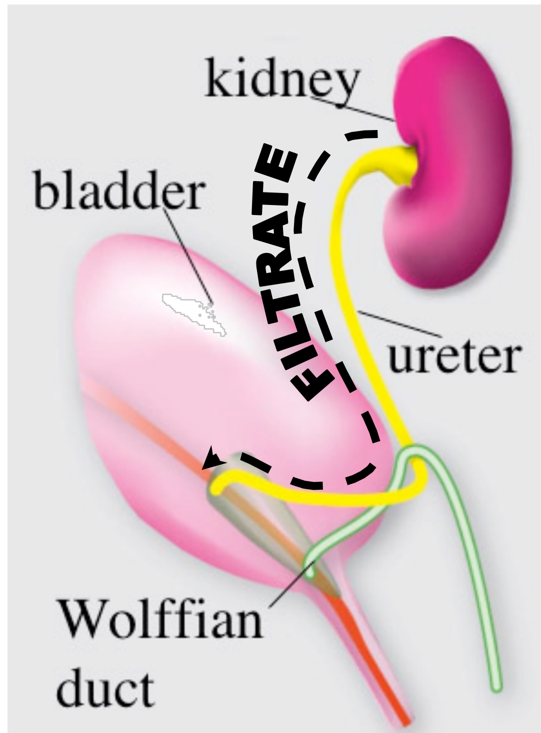
Notch activation: Delta, jagged or serrate ligands on an adjacent cell bind Notch.
The intracellular domain of Notch is cleaved, goes to the nucleus and induces transcriptional activation of Notch target genes.



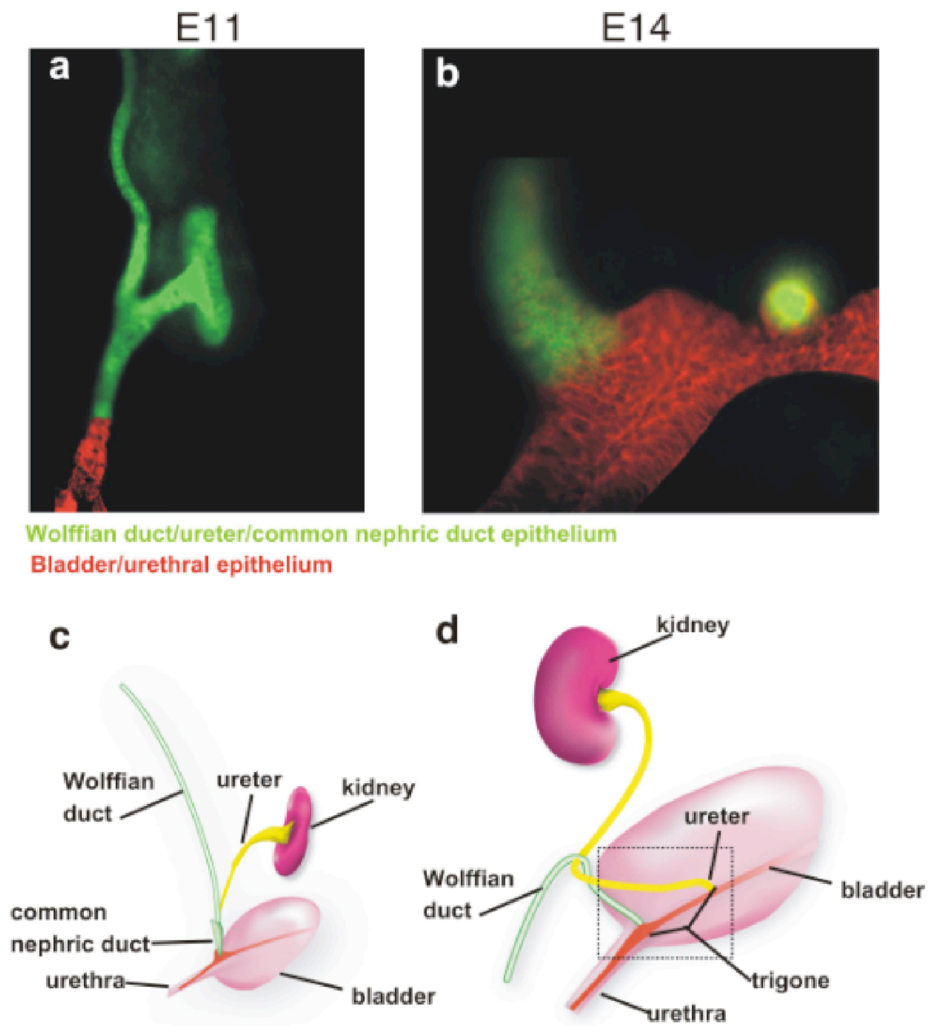
Cheng et al, 2007

Notch2 is expressed in the developing nephron;
Glomerular differentiation is arrested in Notch 2 mutant mice

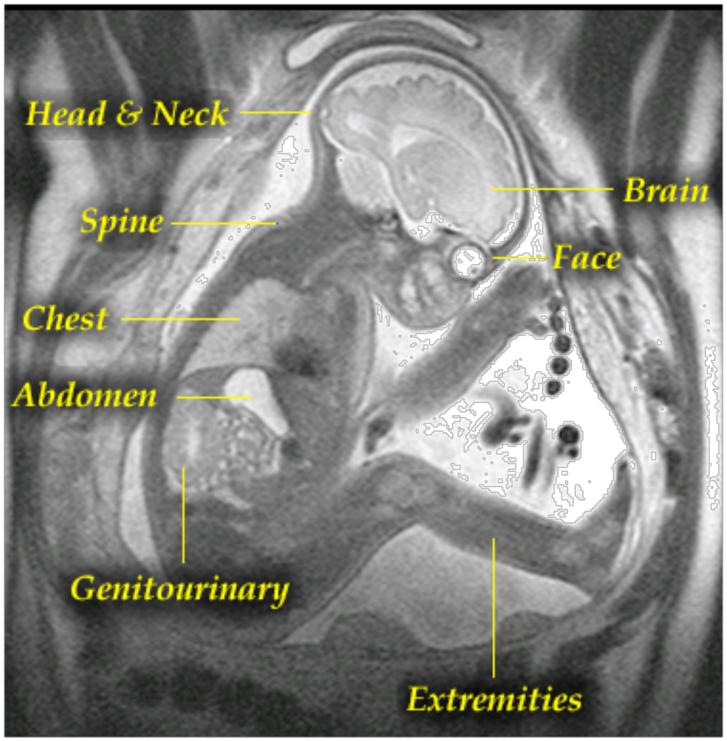
Part II. The lower urinary tract

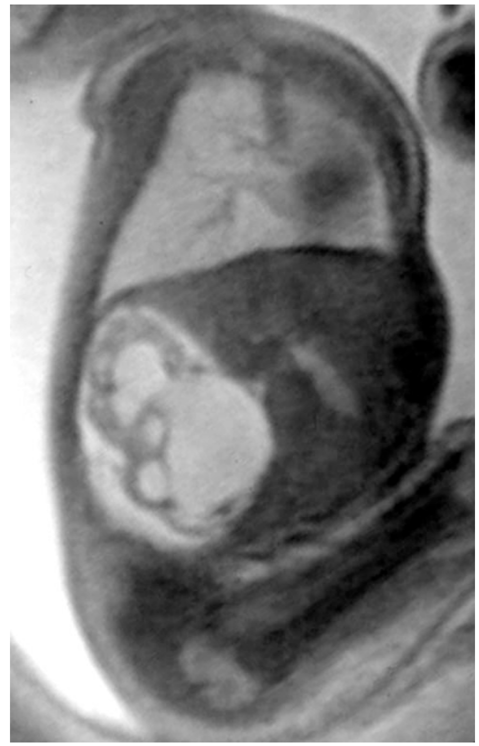


nephrons in the kidney generate urine that is propelled
to the ureters and then to the bladder for storage and excretion



DURING DEVELOPMENT THE URETER MOVES FROM THE WOLFFIAN DUCT TO THE BLADDER



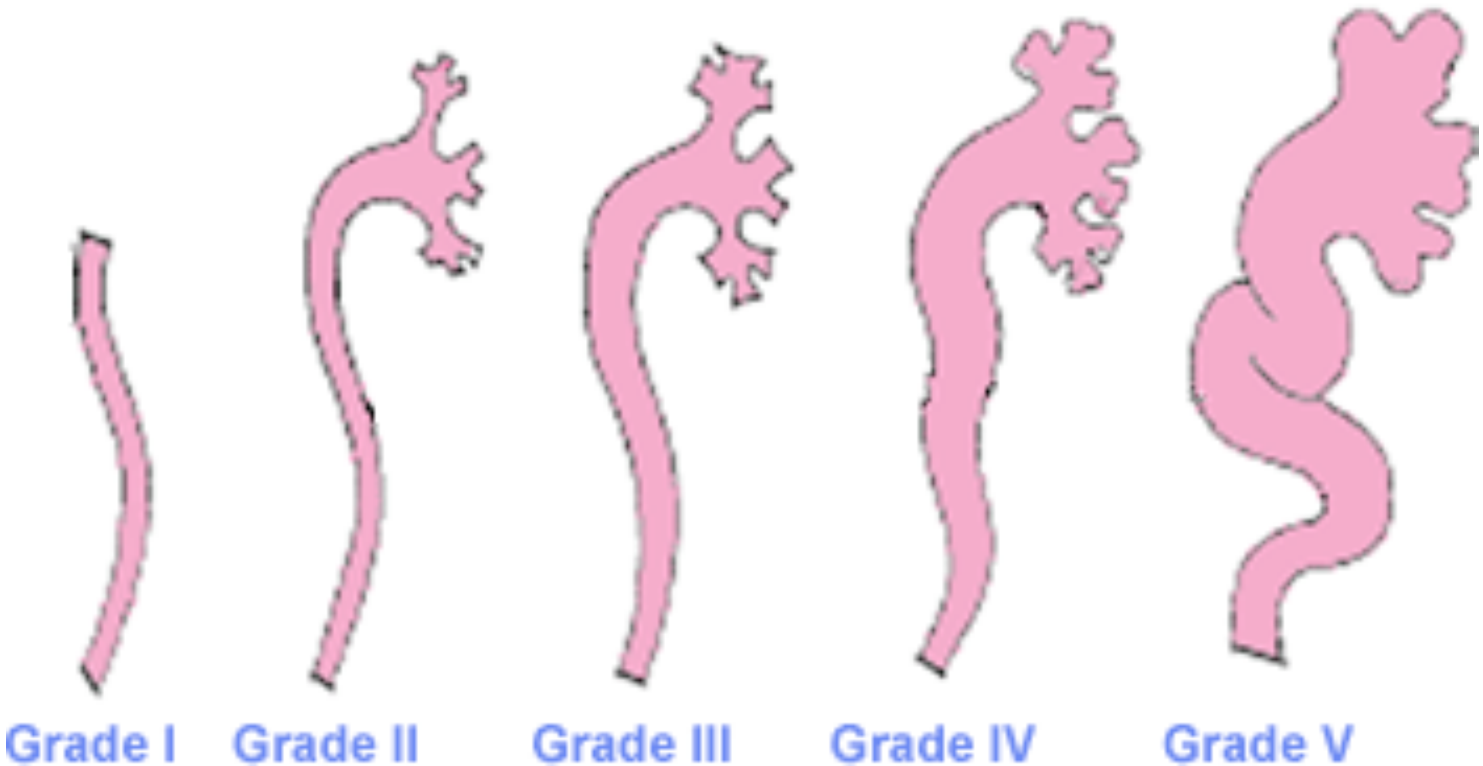


hydronephrosis *in utero*

Defective ureter insertion can cause obstruction and damage the kidney

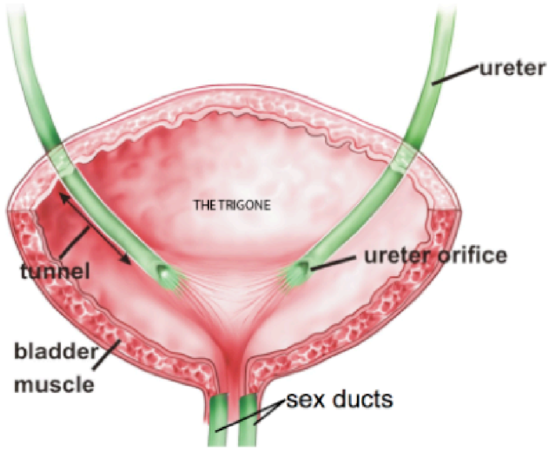
International Reflux Classification

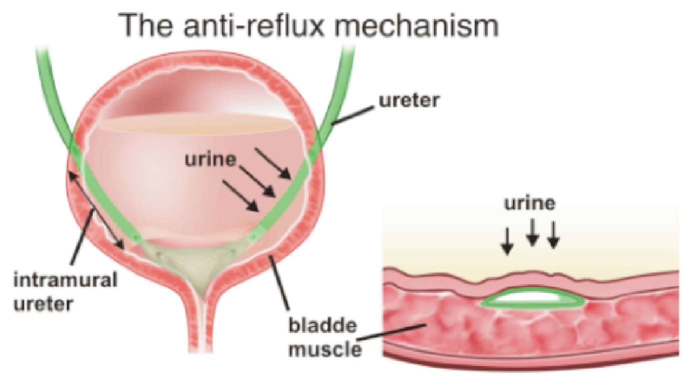
Color indicates the degree of reflux up the ureter and the calyces (i.e. the area where urine collects in the kidney).

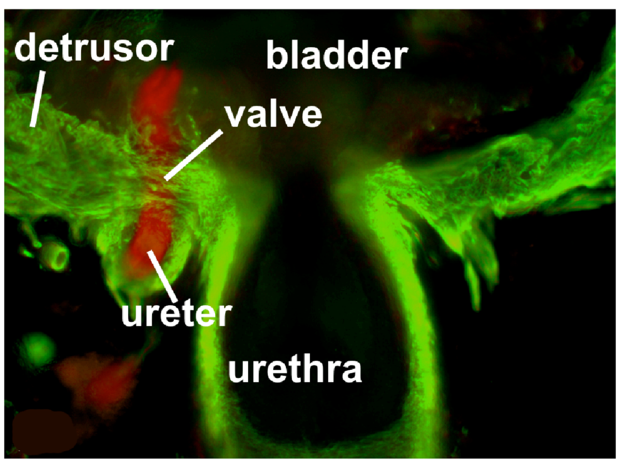


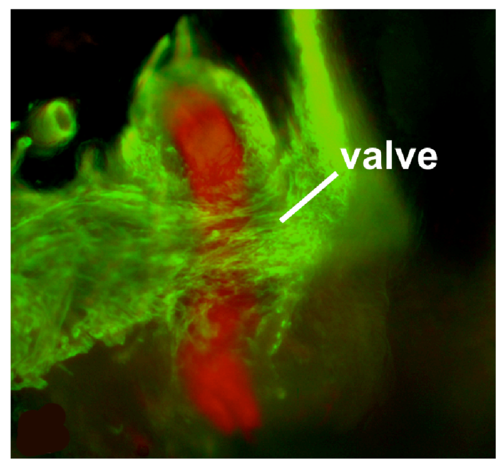
Severe reflux can cause end stage renal disease

The trigone is the site of the anti-reflux mechanism

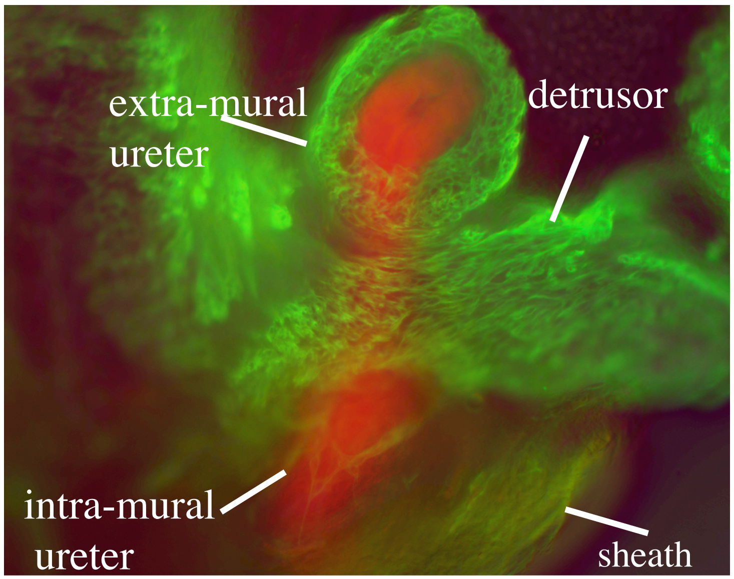




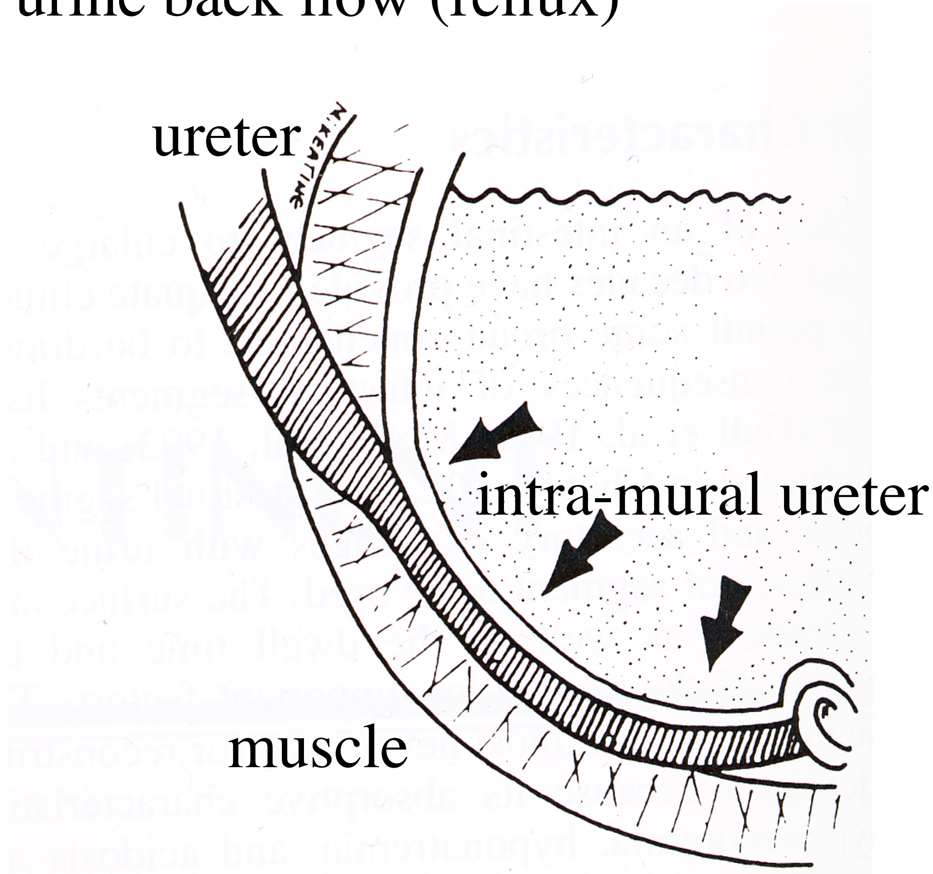




the **ureteral valve** is part of the trigone and is an **anti-reflux** mechanism that prevents urine back flow (reflux)

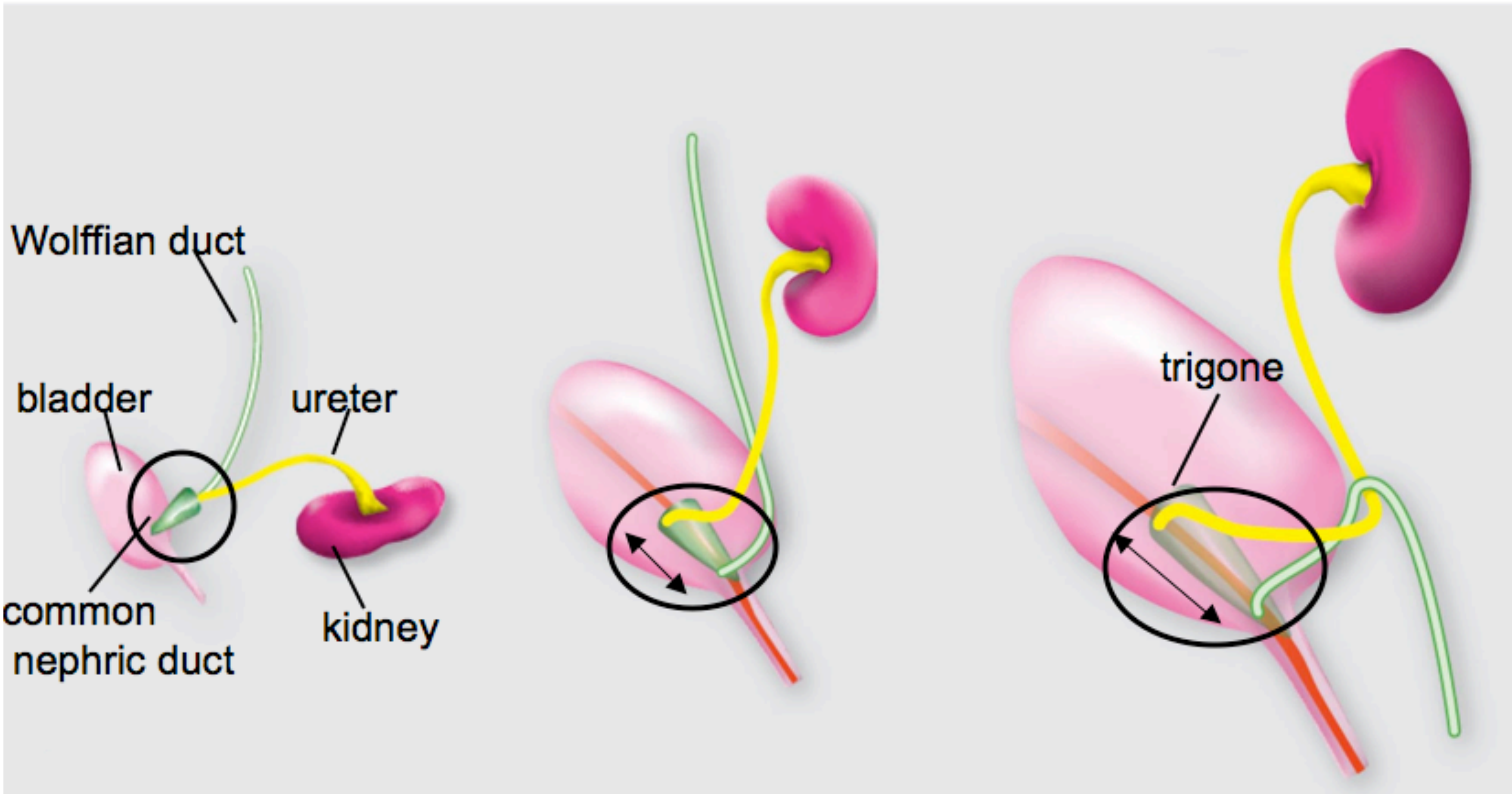


smooth muscle
ureter epithelium
actin



Ureterl valve function depends on insertion of the ureter orifice at the proper position in the bladder neck (trigone)

The trigone contains an anti-reflux valve



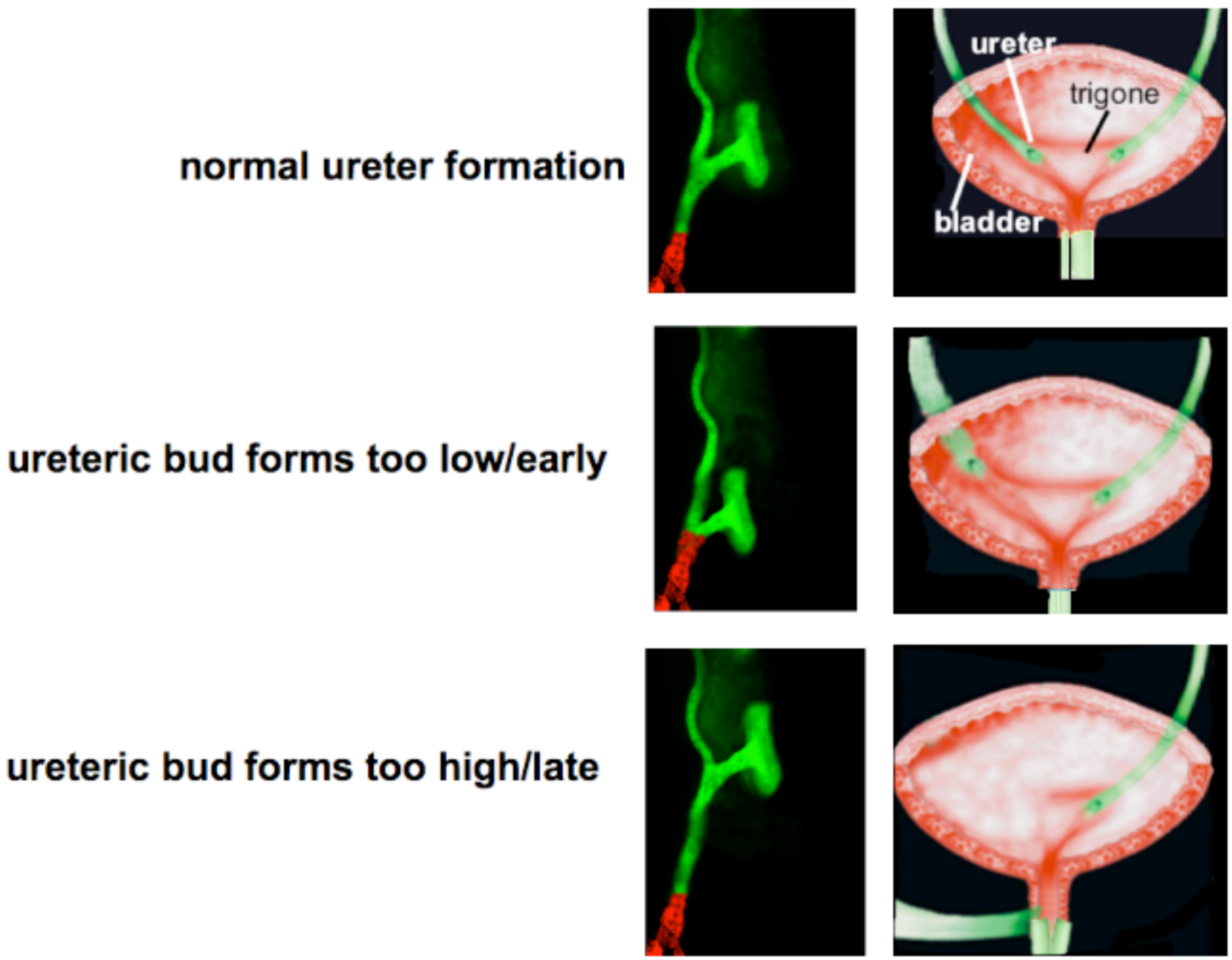
THE **TRIGONE** IS MORPHOGLOGICALLY DISTINCT FROM THE BLADDER AND IS THOUGHT TO BE DERRIVED FROM THE COMMON NEPHRIC DUCT

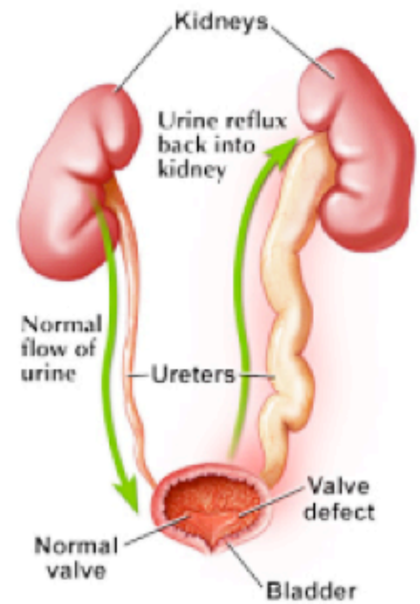
Accepted model of ureter transposition

formation of the **trigone** from the **common nephric duct** repositions the ureters in the bladder

Larsen's Embryology

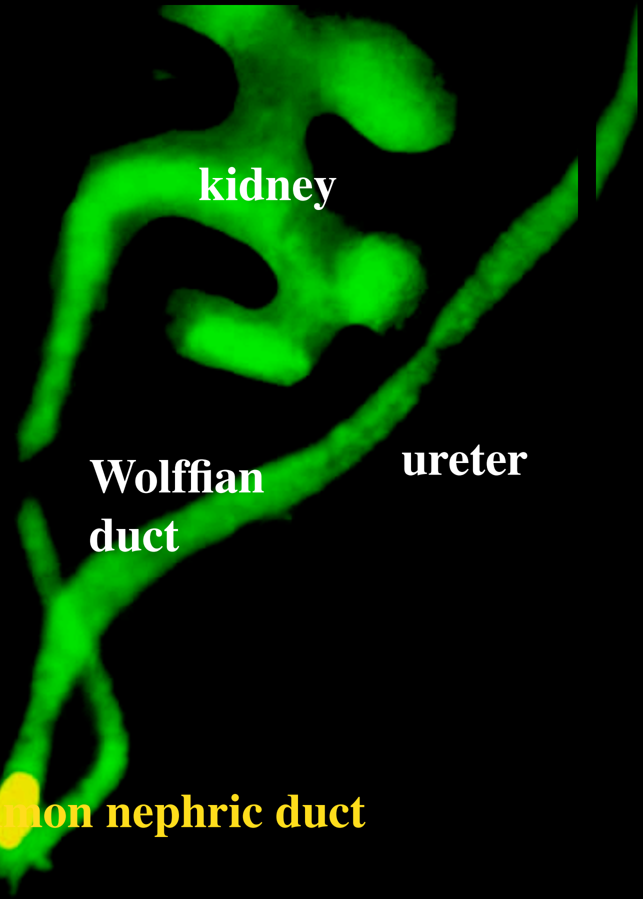
Abnormal connections between the ureter orifice and trigone are associated with vesicoureteral reflux and obstruction



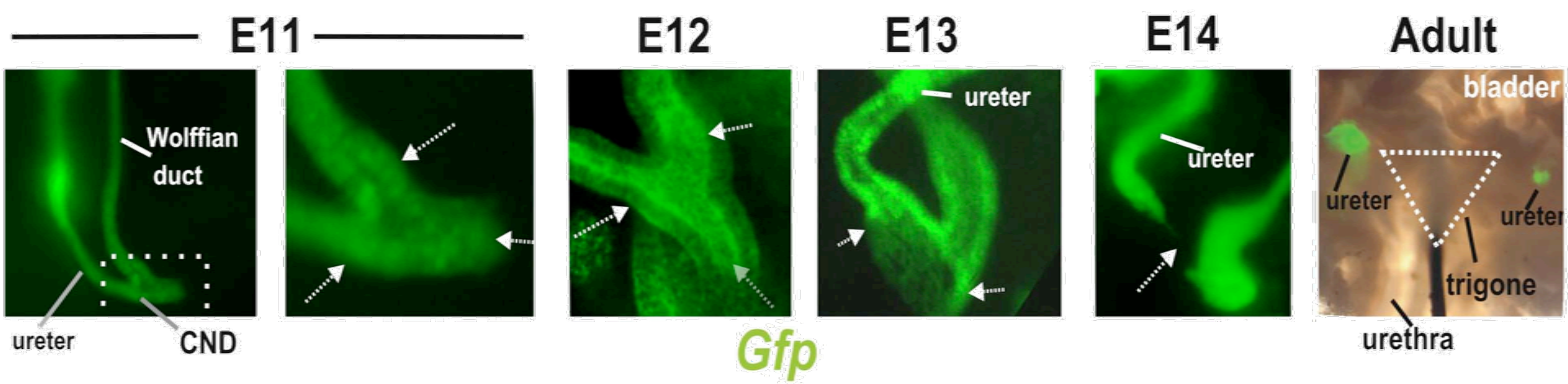


Mackie-Stephens hypothesis: the final position of the ureter with respect to the Trigone depends on the site of its formation on the Wolffian duct

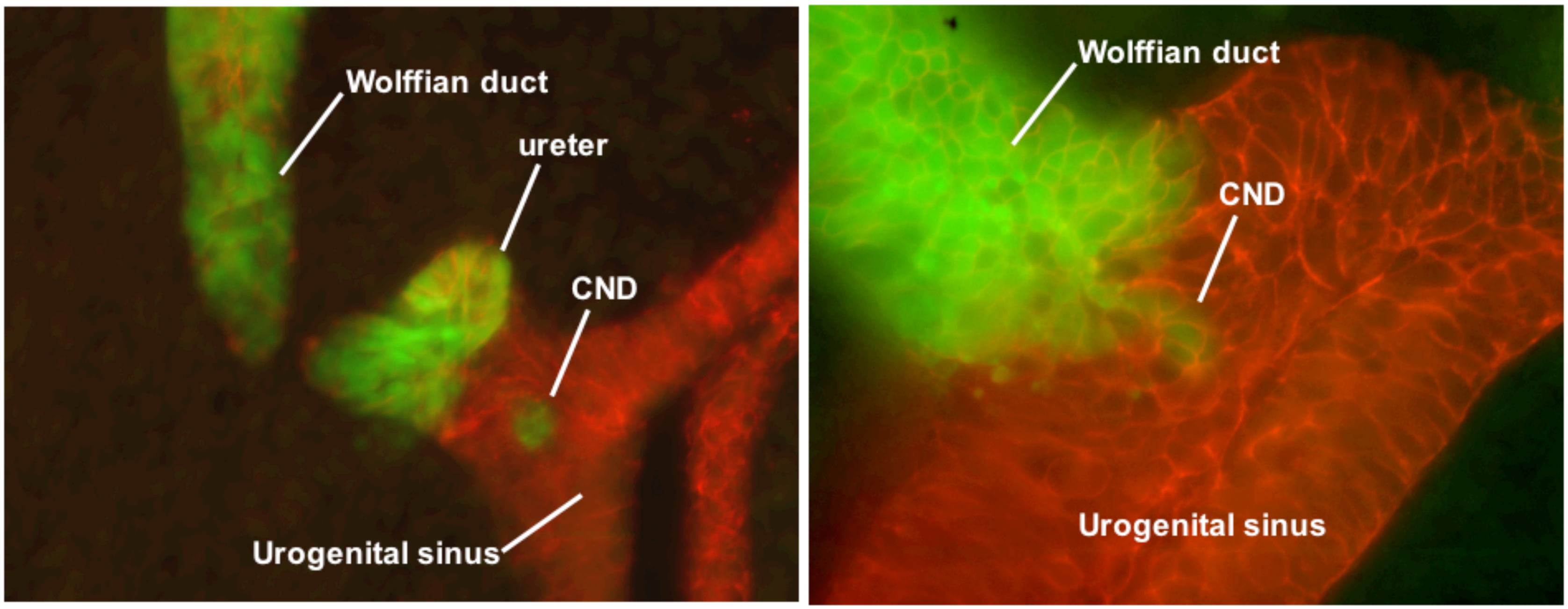
Test the Mackie Stephens hypothesis experimentally using mouse models to re-assess the mechanism of ureter transposition:



expression of green fluorescent protein in the mouse common nephric duct enables us to follow its fate during ureter insertion what happens to the common nephric duct during ureter transposition?



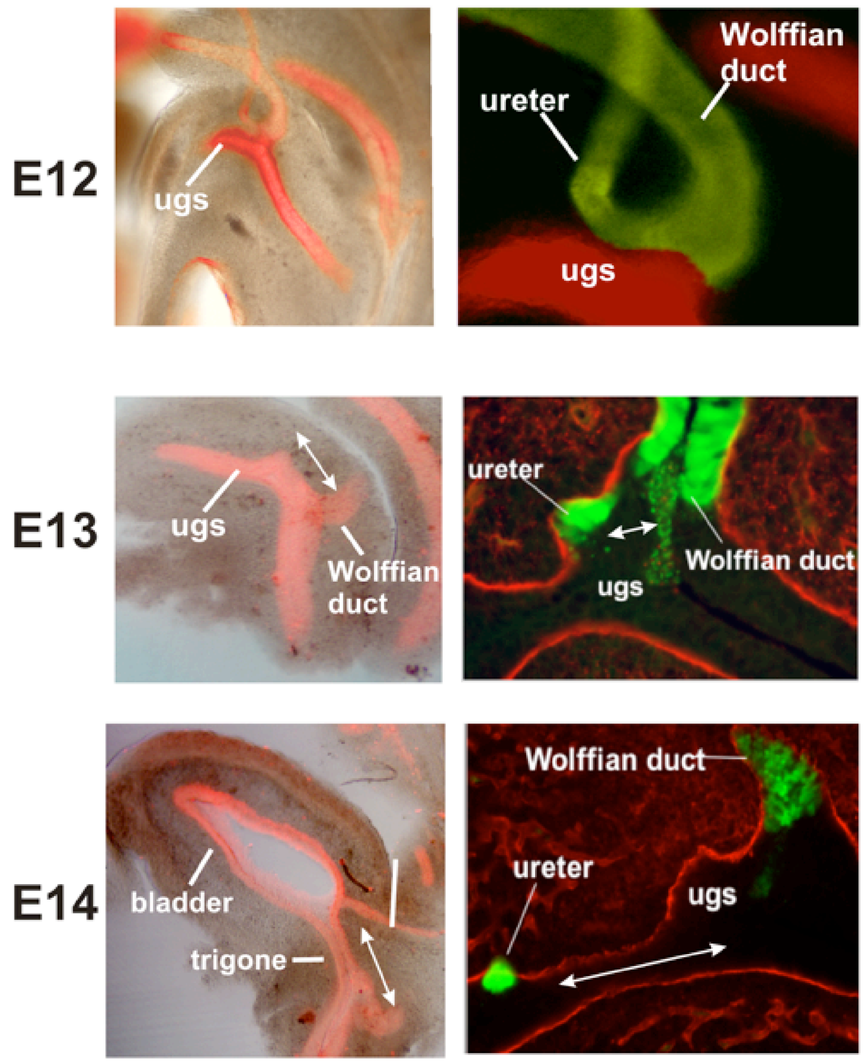
The common nephric duct appears to regress rather than expand



CND cells are absorbed into the urogenital sinus epithelium

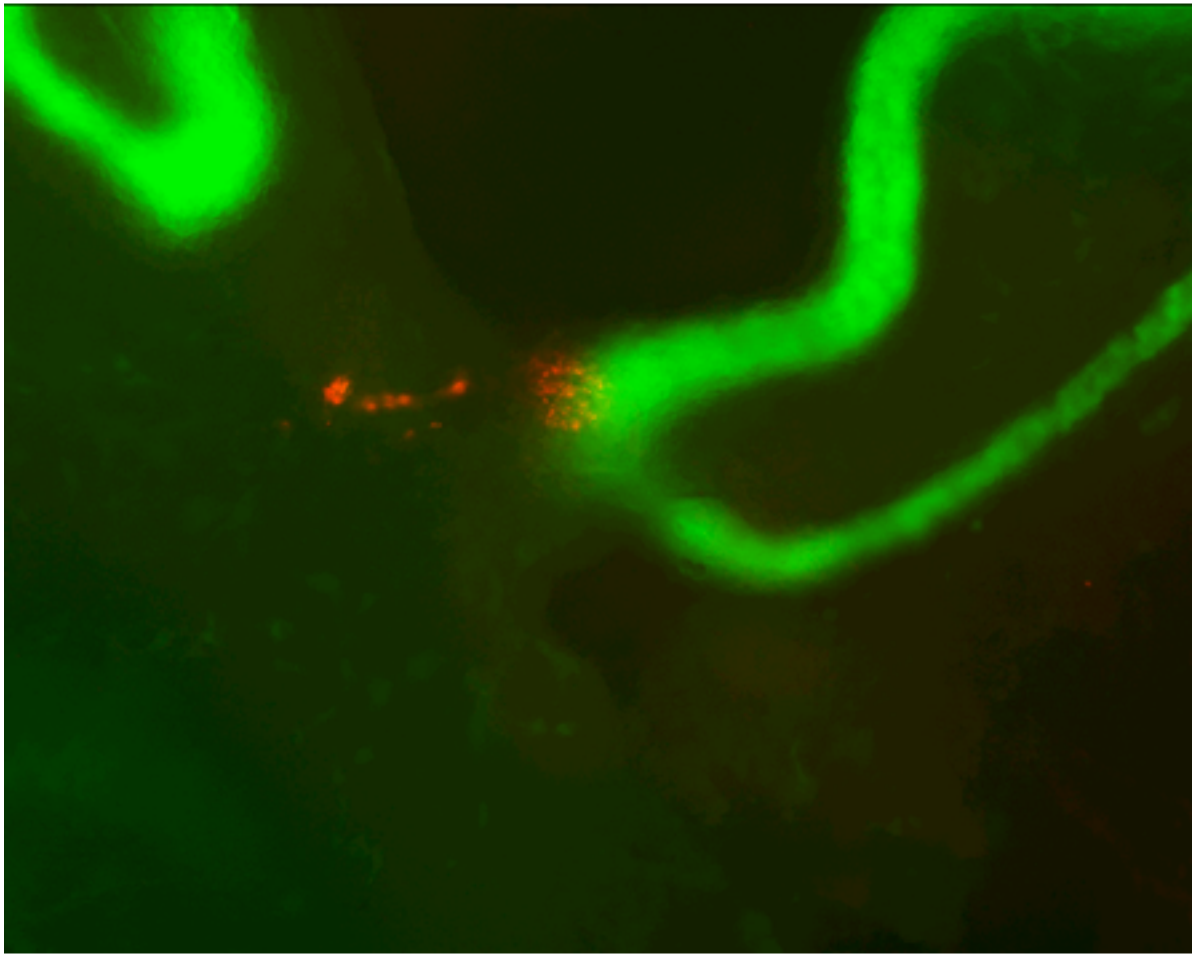
THE CND UNDERGOES APOPTOSIS AND IS UNLIKELY TO FORM THE BLADDER TRIGONE

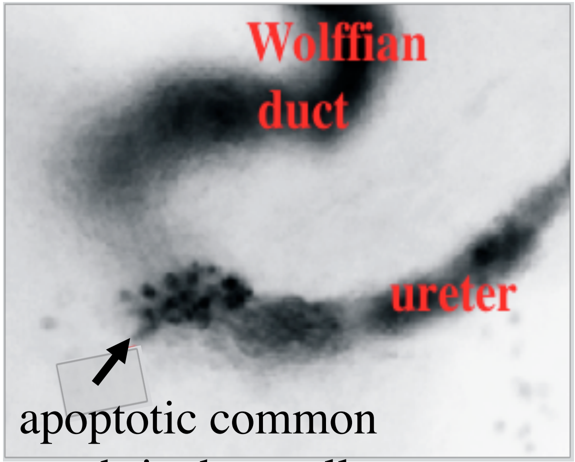
APOPTOSIS OF THE CND ENABLES THE URETER TO SEPARATE AND REPOSITION IN THE BLADDER



ONCE THE CND UNDERGOES APOPTOSIS THE URETER ORIFICE FUSES WITH THE BLADDER EPI AND IS MOVED TO ITS FINAL INSERTION SITE AS THE BLADDER EXPANDS

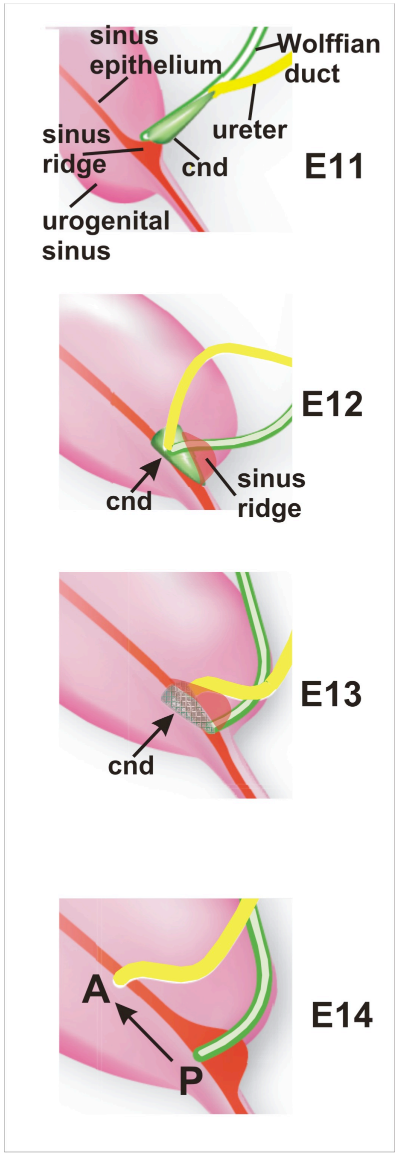
Ureter transposition depends on apoptosis of the
common nephric duct





apoptotic common nephric duct cells

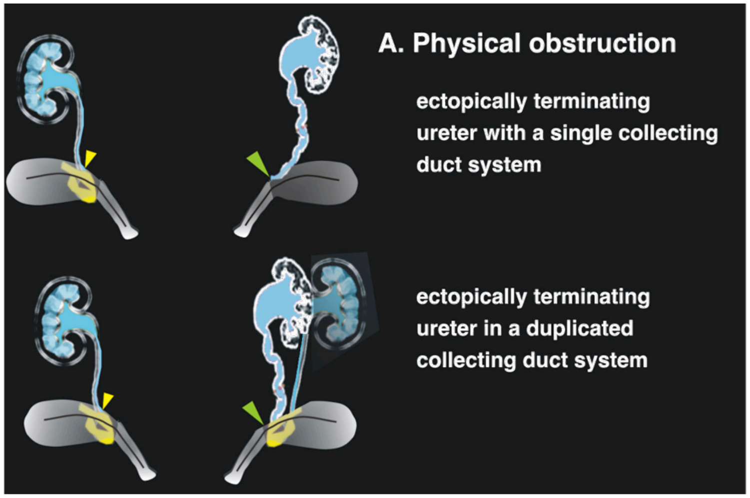
A revised model of ureter transposition



the common nephric duct is absorbed into the expanding urogenital sinus. The ureter makes direct contact with and inserts into the urogenital sinus

apoptosis of the common nephric duct enables the ureter orifice to detach from the Wolffian duct

continued growth and expansion of the urogenital sinus moves the ureter orifice further anterior to the bladder neck

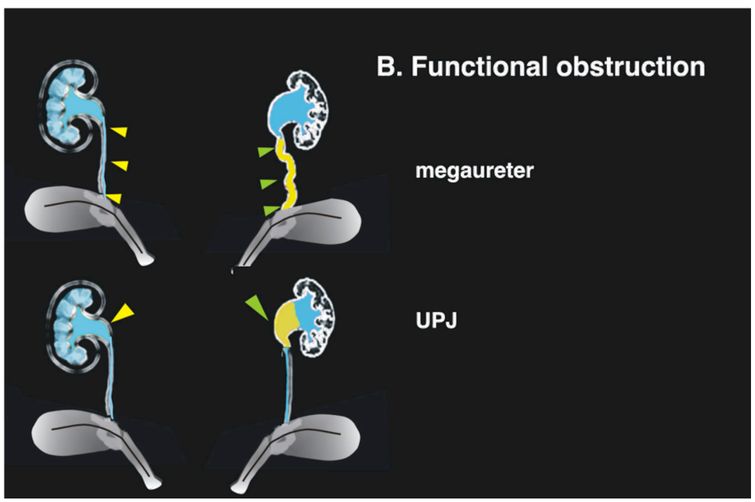


abnormal position of the ureter orifice

Impaired retinoid signaling, Ret

sprouty, slit-2, retinoid excess

Physical vs Functional obstruction



abnormal peristalsis

sonic hedgehog (muscle)

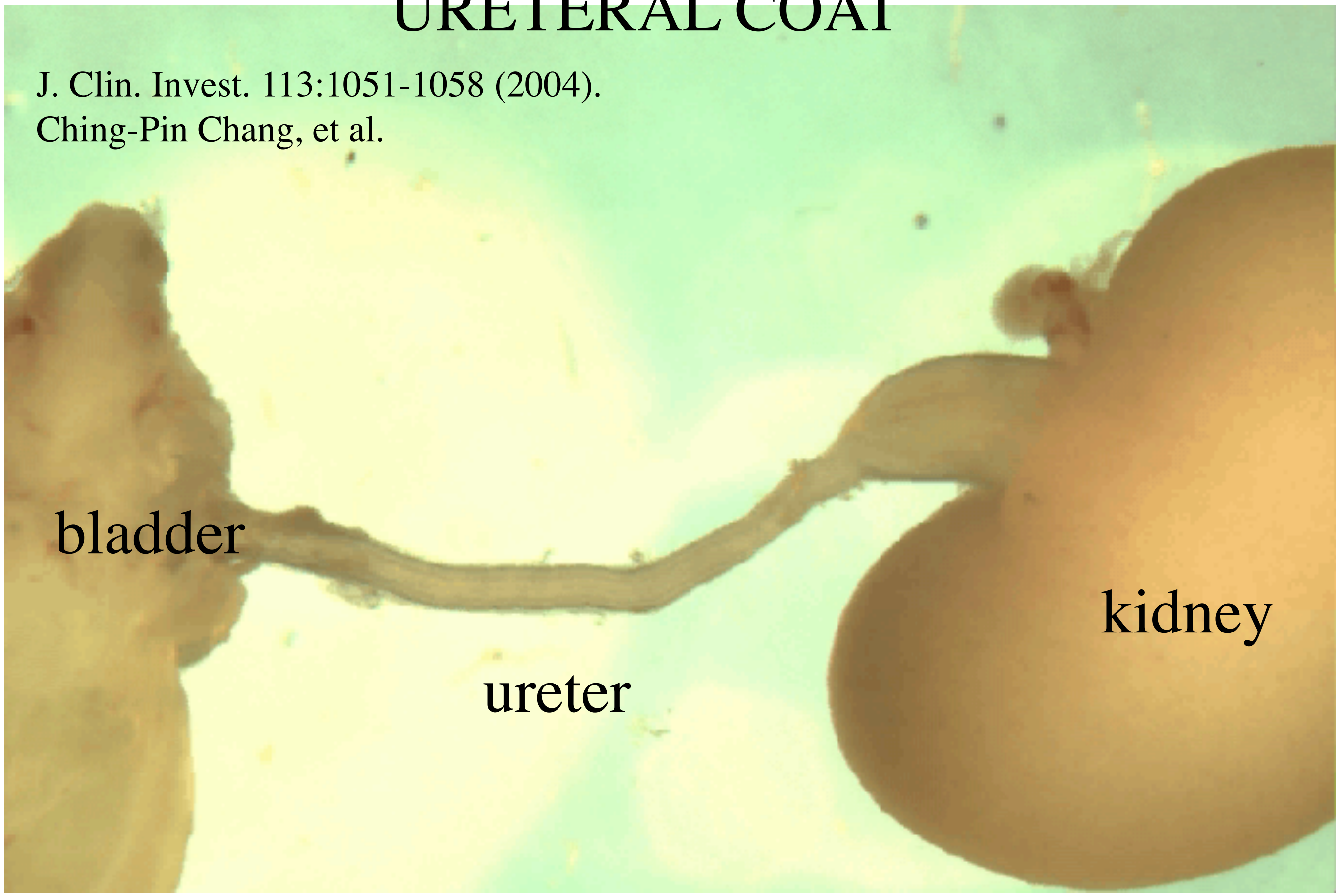
Calcineurin B (peristalsis)

uroplakin (epithelium)

Tbx18

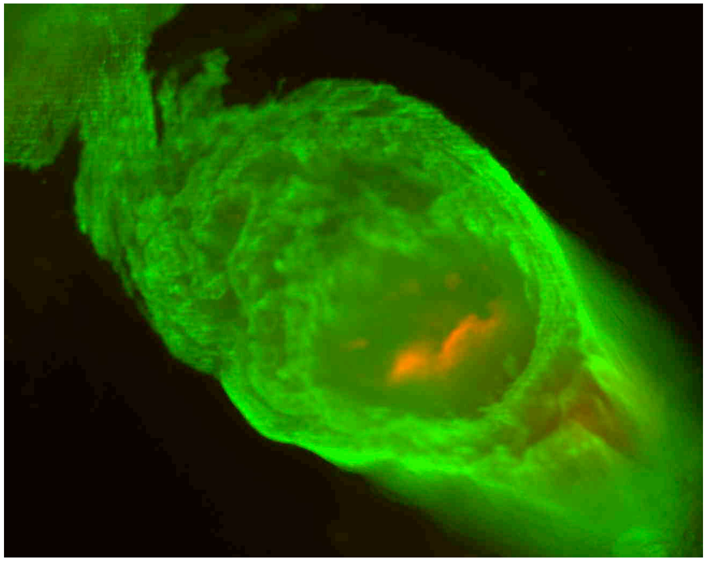
Intrinsic ureteral abnormalities can cause obstruction

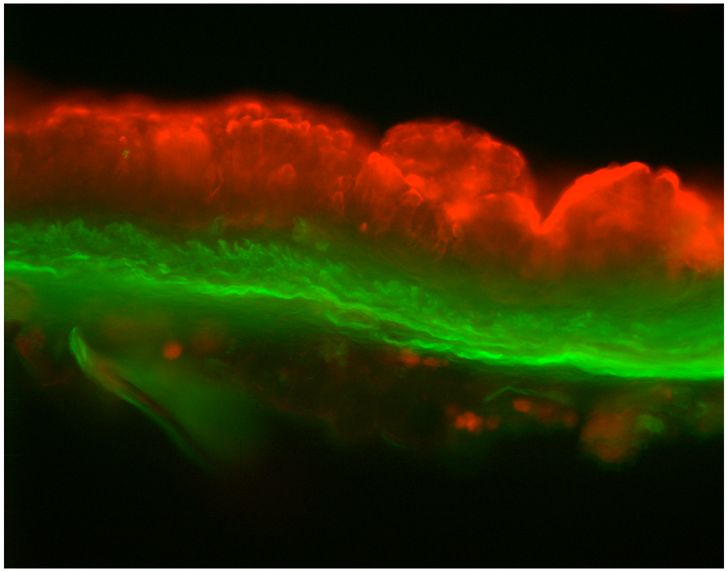
URETER PERISTALSIS IS MYOGENIC, MEDIATED BY SM IN THE URETERAL COAT



Impaired peristalsis is a cause of obstruction
(functional obstruction)

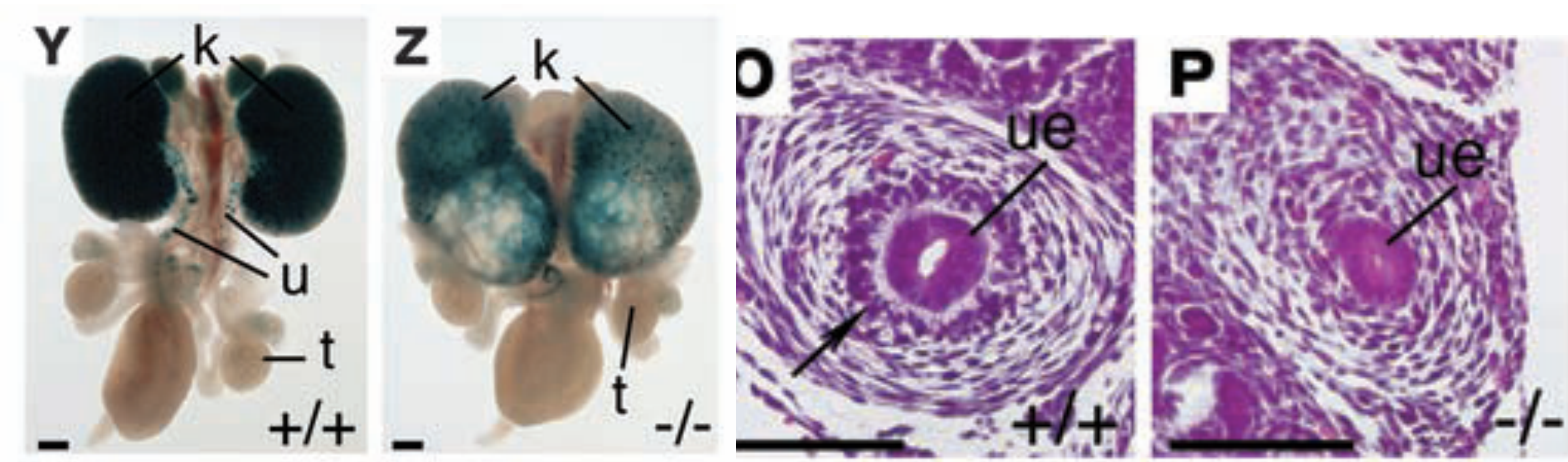
- a transitional epithelium expressing uroplakin lines the ureters
- The ureter smooth muscle coat mediates myogenic peristalsis
- defective smooth muscle formation or mutations in uroplakins cause functional obstruction





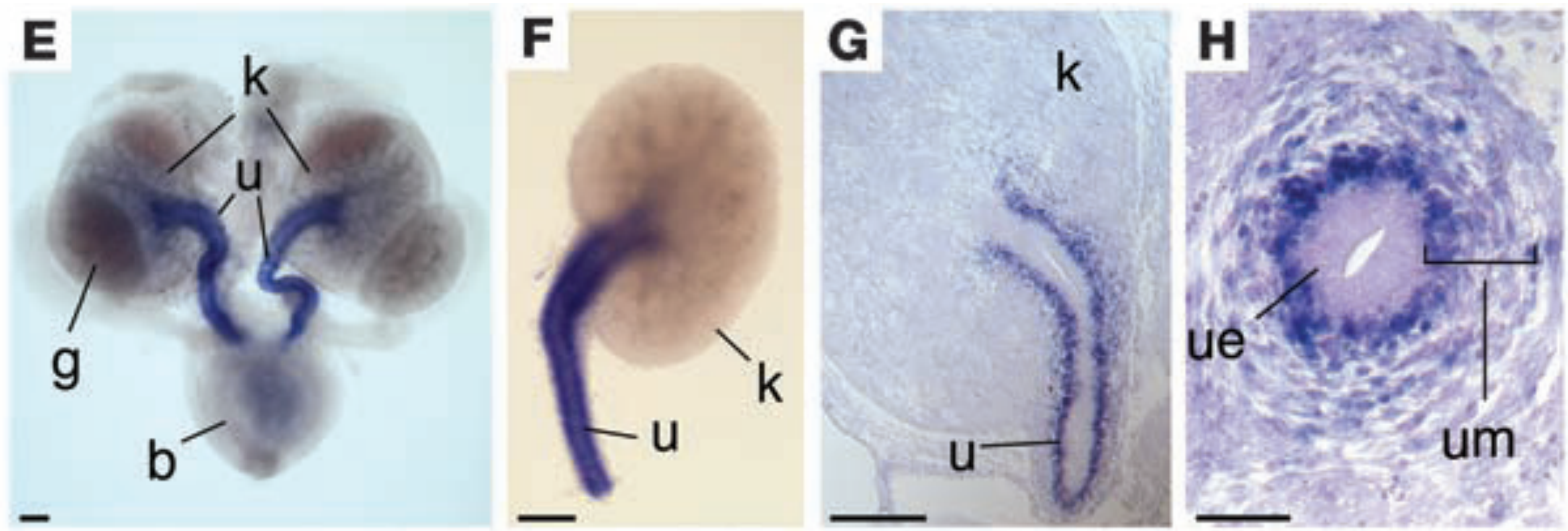
smooth muscle actin
uroplakin

Loss of Tbx18 results in megaureter



Airek et al.

Airek et al.



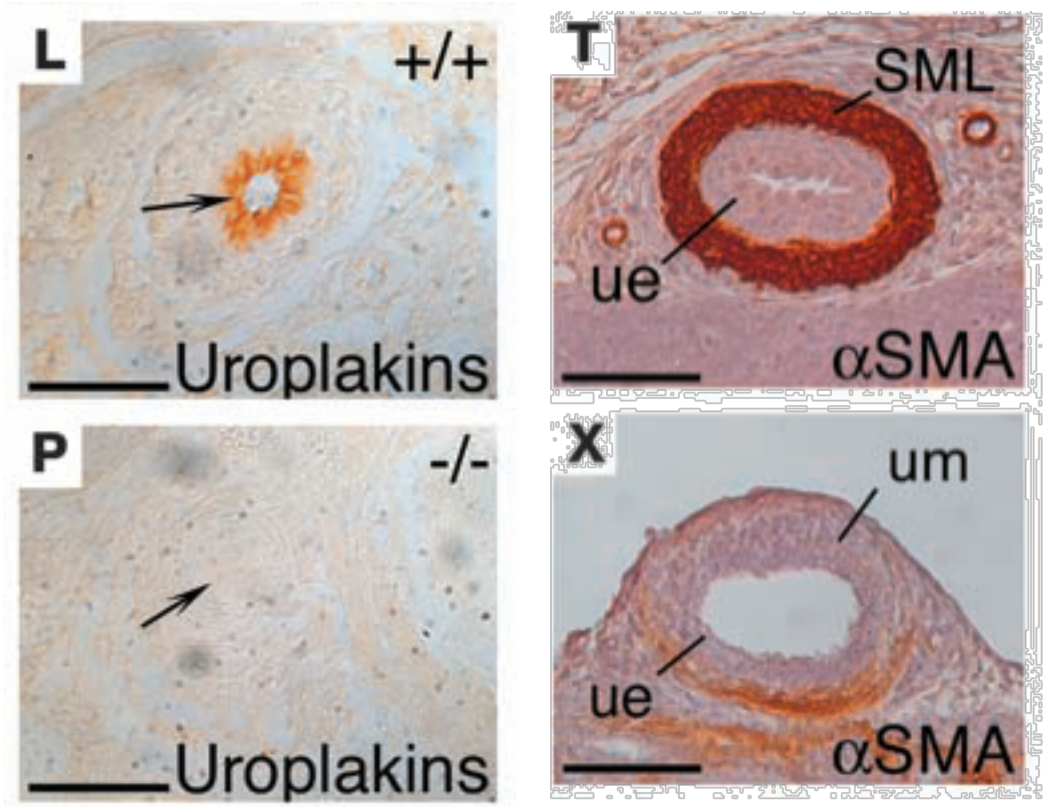
WT Tbx18- WT Tbx18-

Tbx18 is selectively expressed in peri-ureteral mesenchyme where it is required for ureter radial patterning

Loss of Tbx18 expression in periureteral mesenchyme results in smooth muscle defects as well as epithelial abnormalities

wt

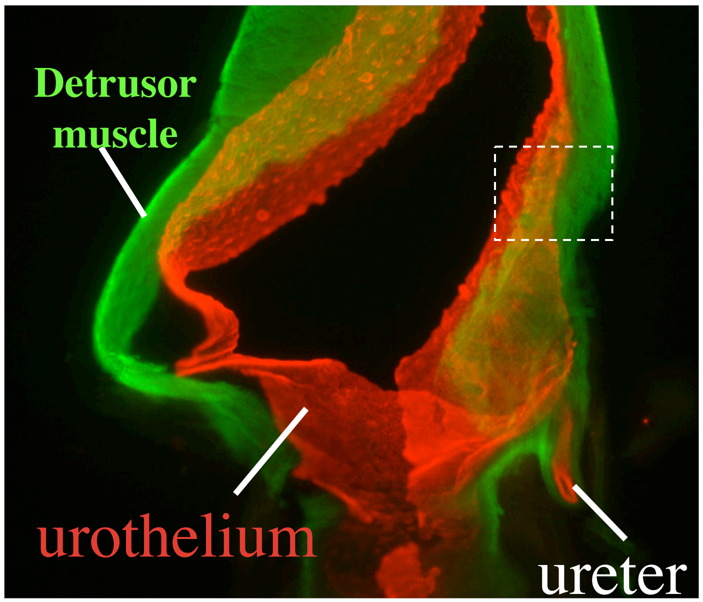
Tbx18-

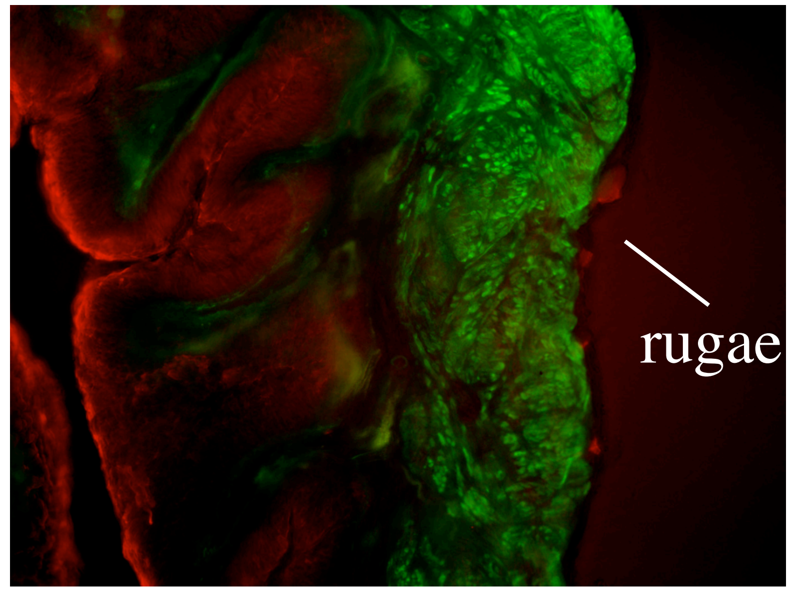


The ureter is radially patterned by epithelial mesenchymal signals

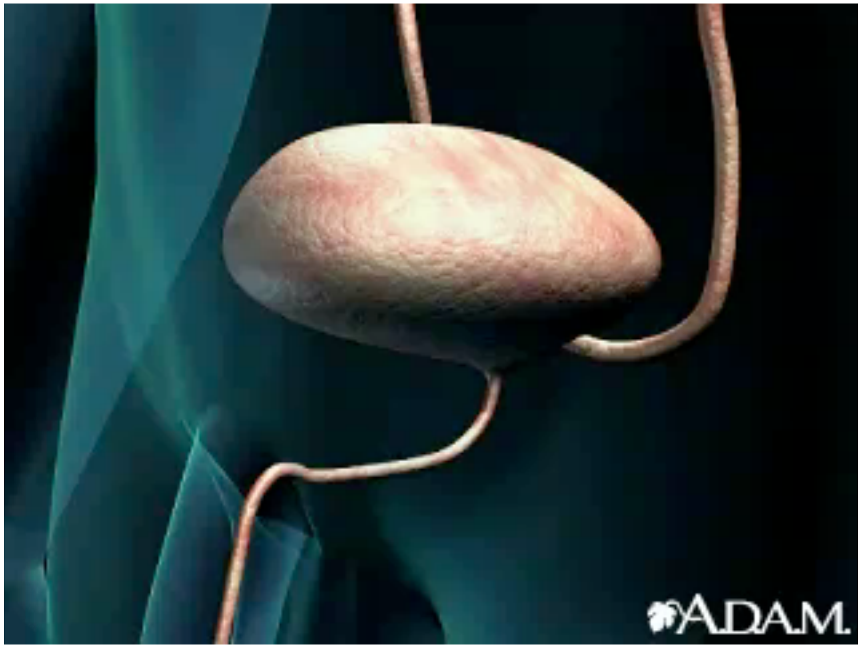
What signaling pathways are important for bladder formation?

The bladder epithelium is lined with
plaques made from **uroplakins** that form a water-proof barrier

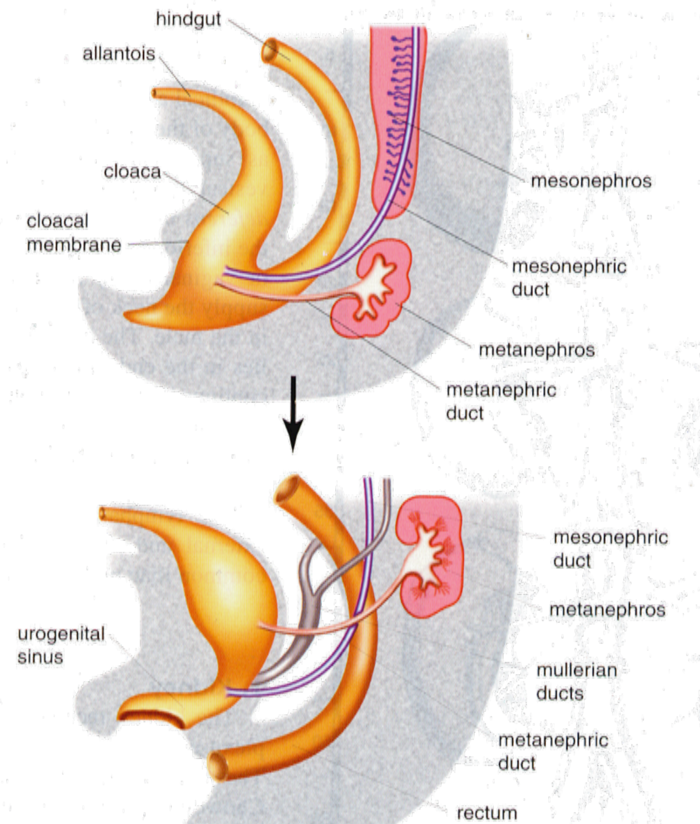




smooth muscle of the detrusor and rugae (folds) in the urothelium
allow the bladder to expand and contract


A.D.A.M.

Larsen's Embryology, 6th Edition



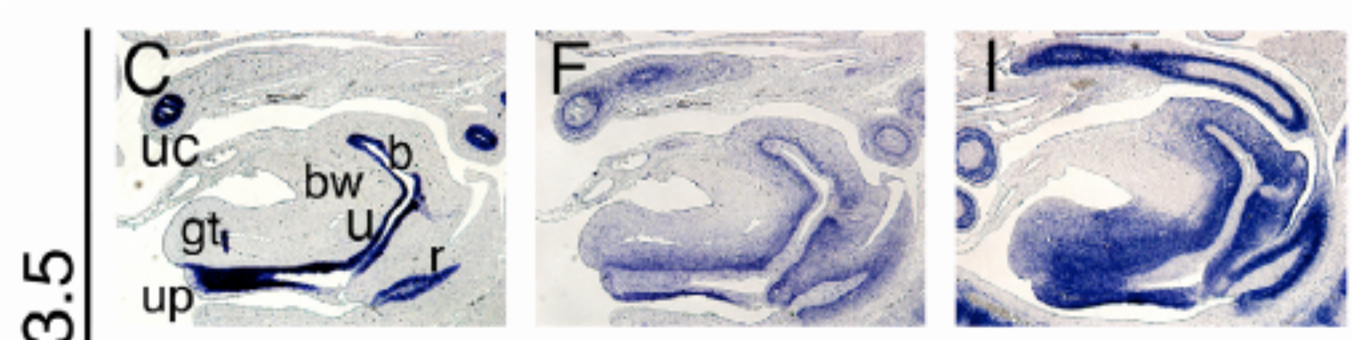
from: The kidney: Eds, Vize et al., 2003)

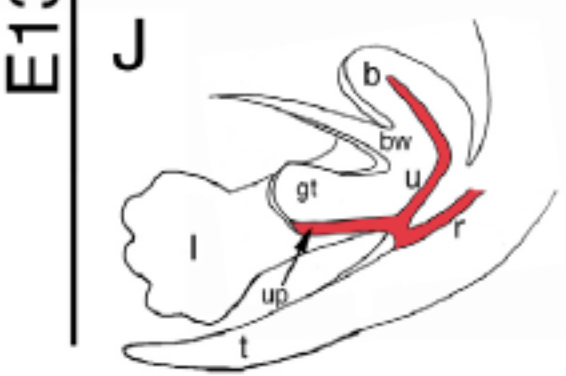
The urorectal septum partitions the cloaca into the urogenital sinus (ventral) and hindgut (dorsal)

The urogenital sinus forms the bladder and urethra in both sexes

Sonic Hedgehog is localized in the bladder/urethral epi
Patched and Gli1, downstream shh targets are localized in bladder/urethral mes

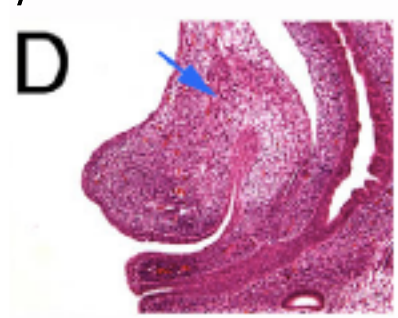
Shh Ptch1 Gli1





wt

Haraguchi et al., 2007



sh
h

Shh is required for bladder formation