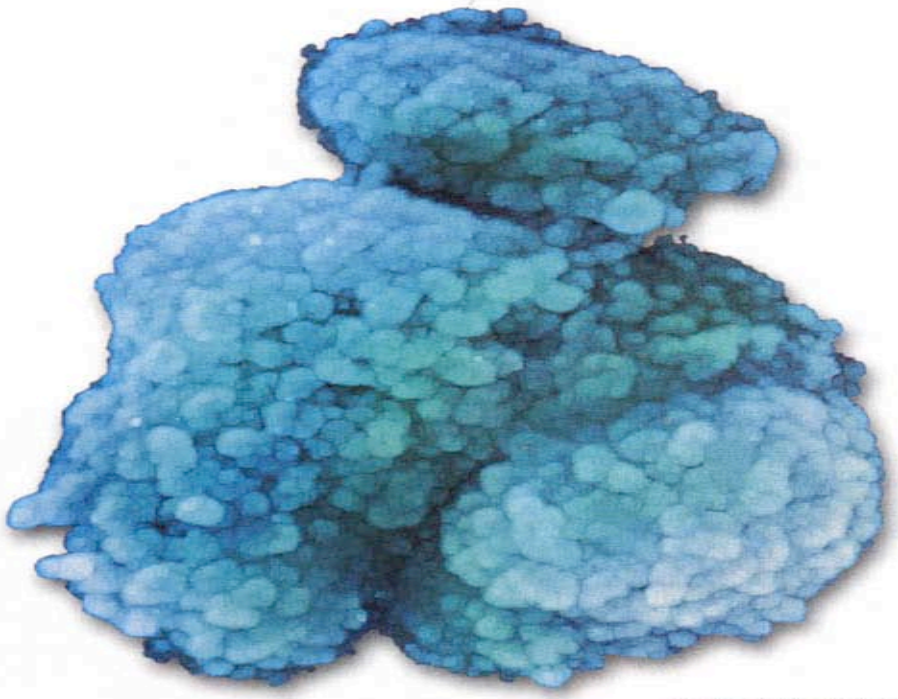
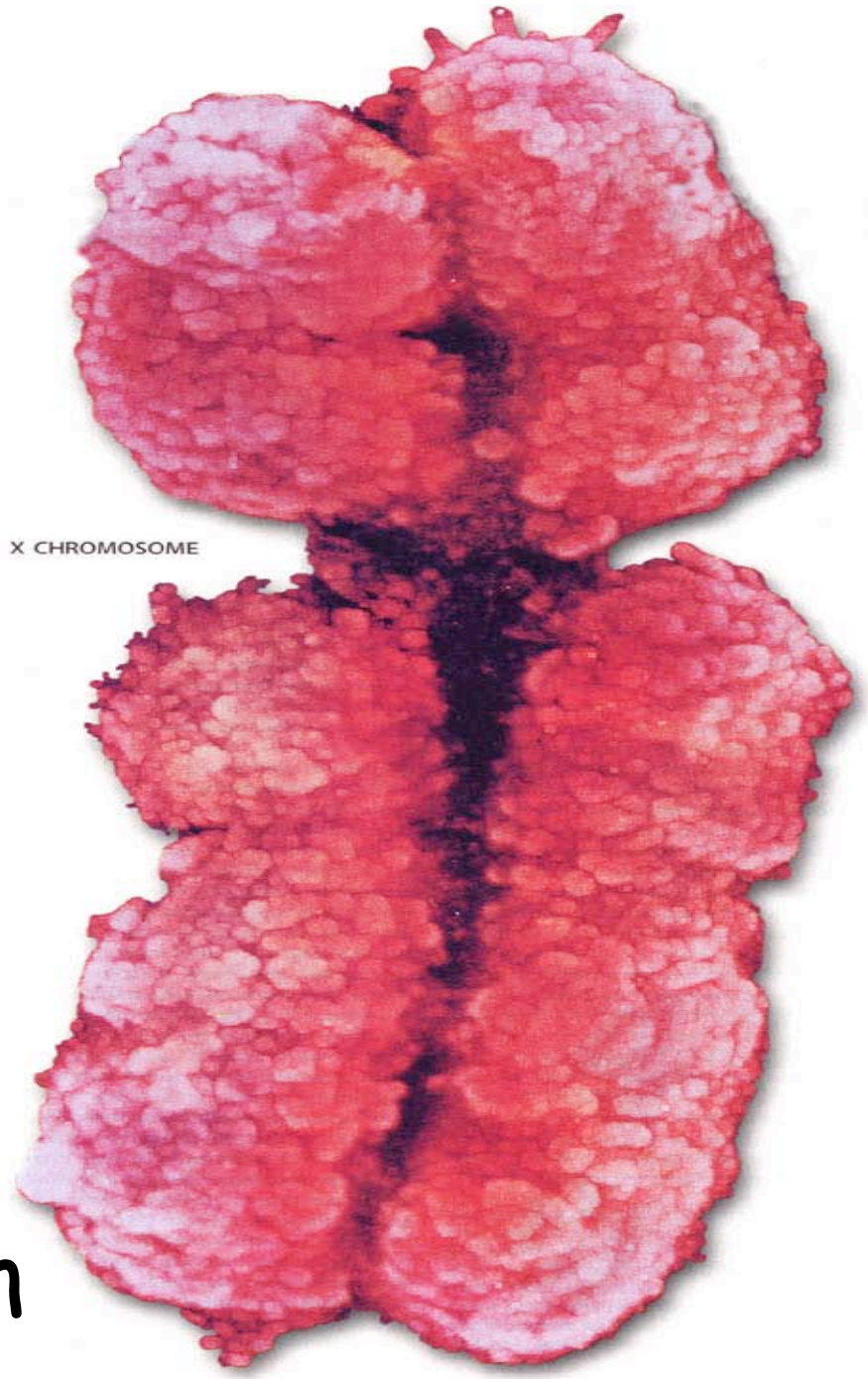


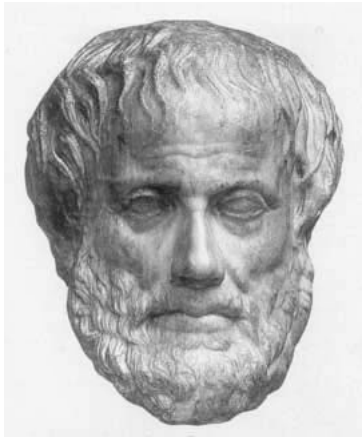
Y CHROMOSOME



X CHROMOSOME

Genetic Sex Determination

Sex is determined by the heat of male partner during intercourse…..



Aristotle (384-322 B.C.)

Courtesy of Humphrey Yao

Sex Differentiation: a favorite topic for philosophers and scientists

8th BC	Homer: Conception is influenced by the wind, north for males and south for females…at least in sheep
130-200 A.D.	Galen: Semen from left testis makes females, right makes males. A mixture produces hermaphrodites.
1677	Anton van Leeuwenhoek: sperm
1827	Carl Ernst von Baer: ovum
1902	Clarence McClung: the "Accessory chromosome"
1947	Alfred Jost: differentiation of the reproductive tract
1949	Barr & Bertram: discovery of the Barr bodies
1959	Welshons & Russell: the role of the Y chromosome
1991	Lovell-Badge et al: discovery of the *SRY* gene

Courtesy of Humphrey Yao

The Jost Paradigm

Genetic Sex Determination

Environmental Sex Determination

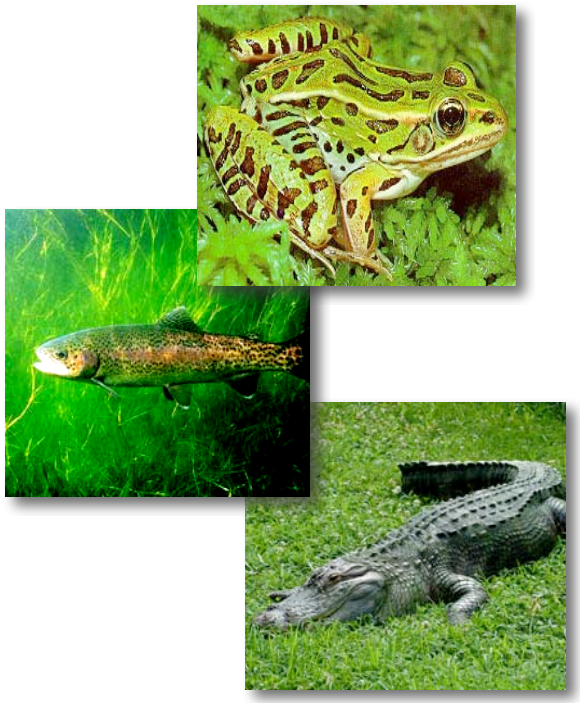
Sex Chromosomes

Switches

Temperature, social cues, etc

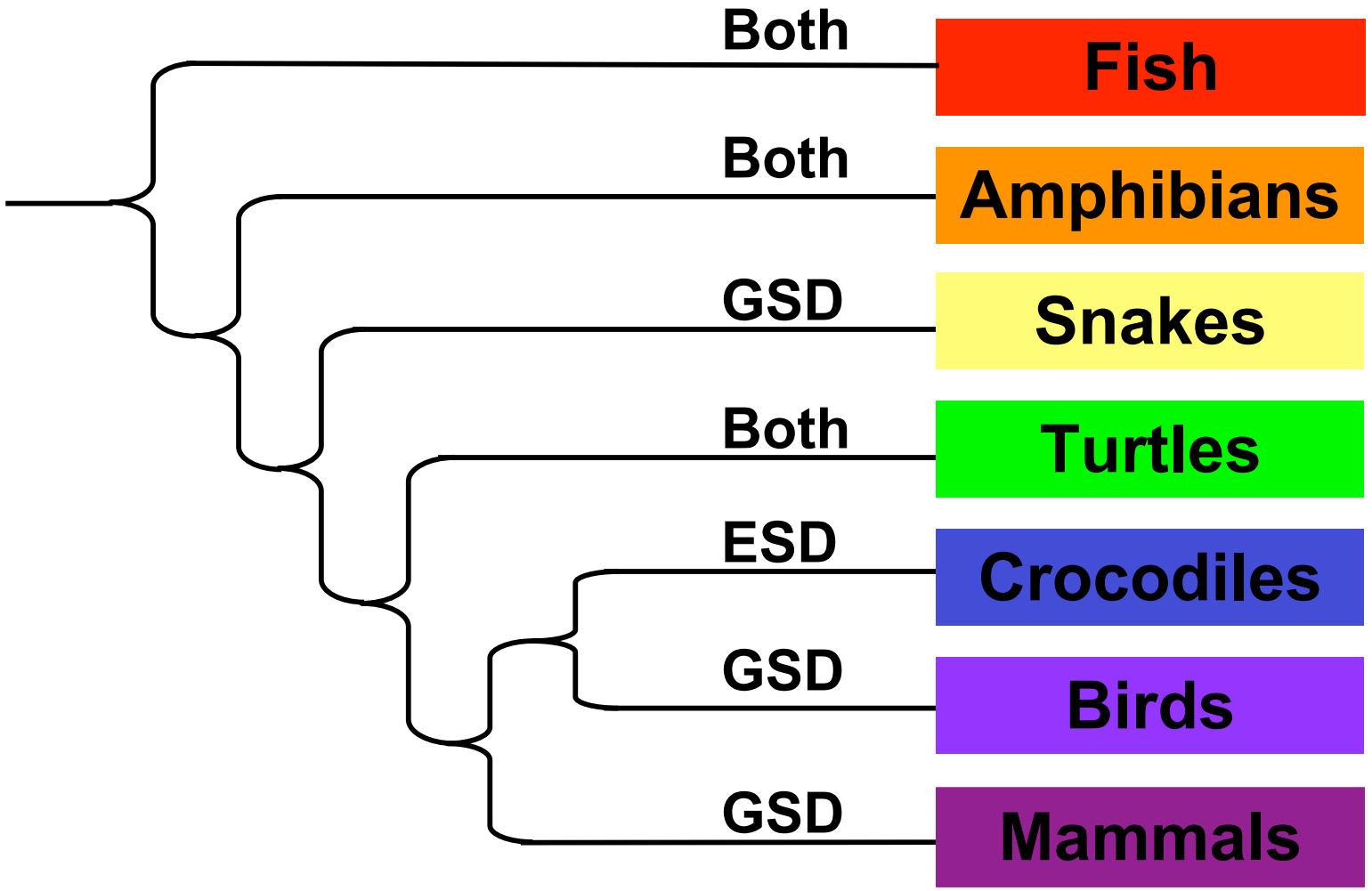
Gonadal Sex

Phenotypic Sex



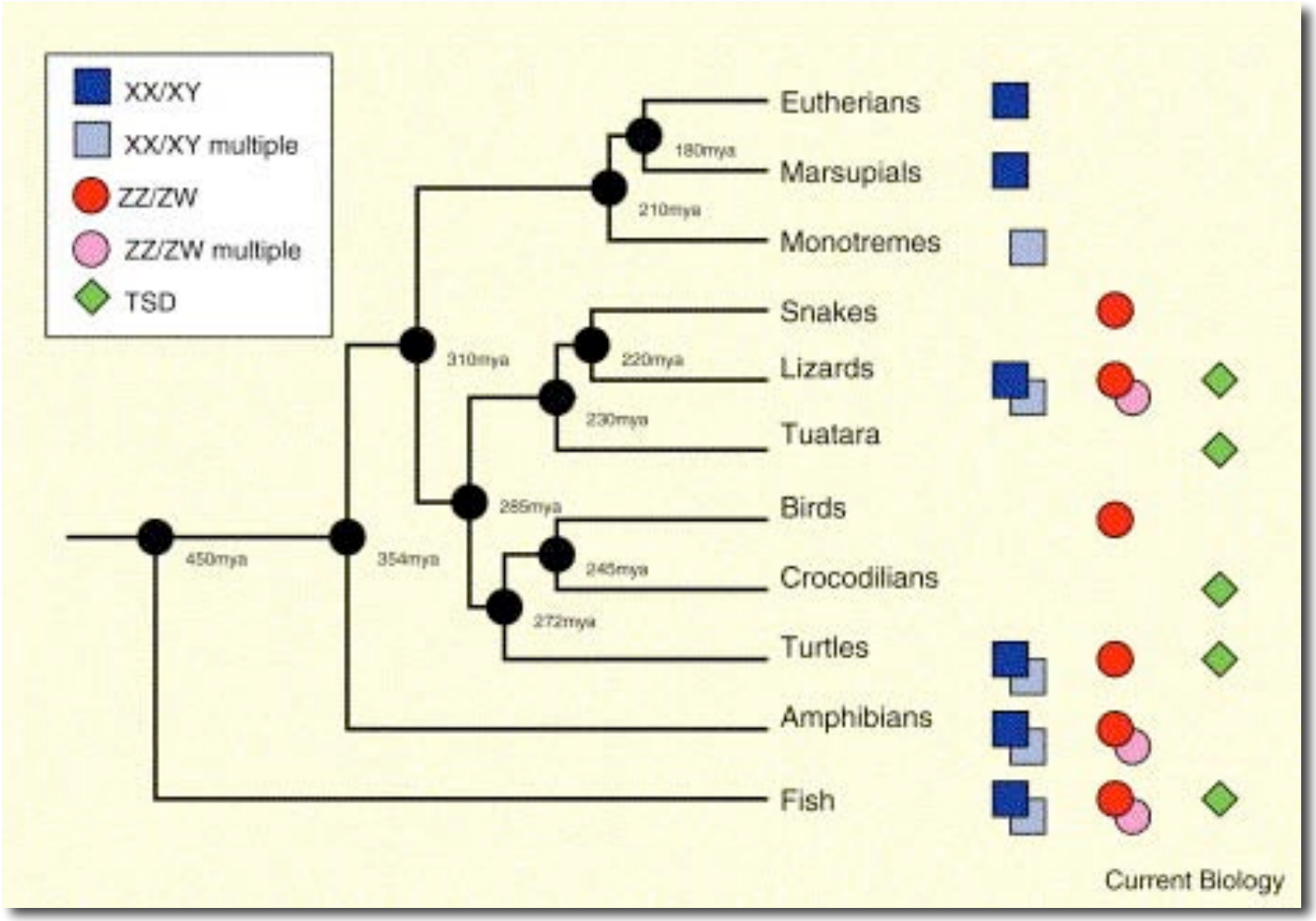
Courtesy of Humphrey Yao

Evolution of Sex Determination Mechanisms



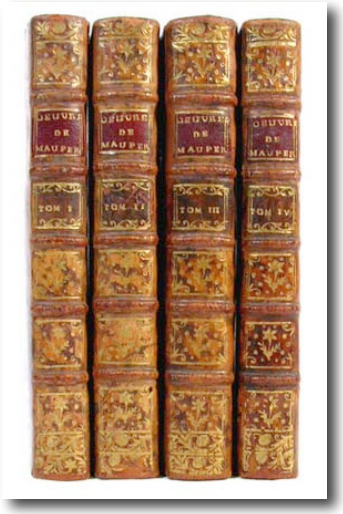
Courtesy of Humphrey Yao

Evolution of Sex Determination Mechanisms



Sex Determination

- *Earthly Venus* - offspring of different races
  - Pierre Louis Moreau de Maupertuis
  - Venus physique / The Earthly Venus
  - La Haye, 1745



- Speculation on organismal adaptation to environment 100 yrs before Darwin
- *Examined debate on source of humans*
  - Sperm
  - Egg
- How did he test this?
  - Hint: della Porta

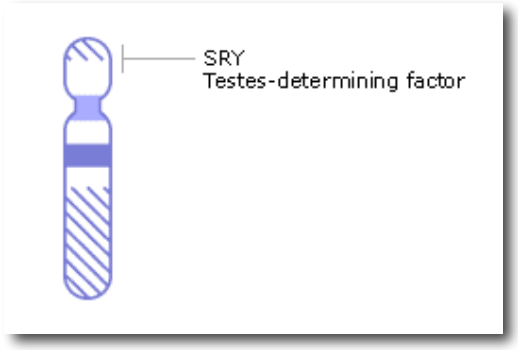
Genetics of Sex Determination

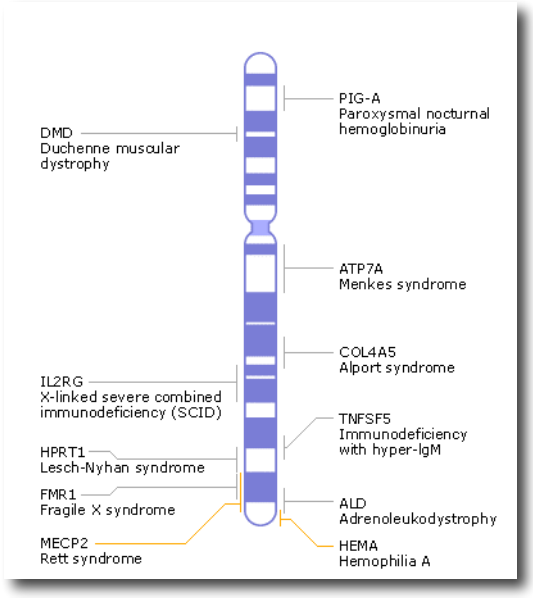
- rediscovery of Mendel /others suggested genetic factor

1902 **Clarence McClung: the "Accessory chromosome"**

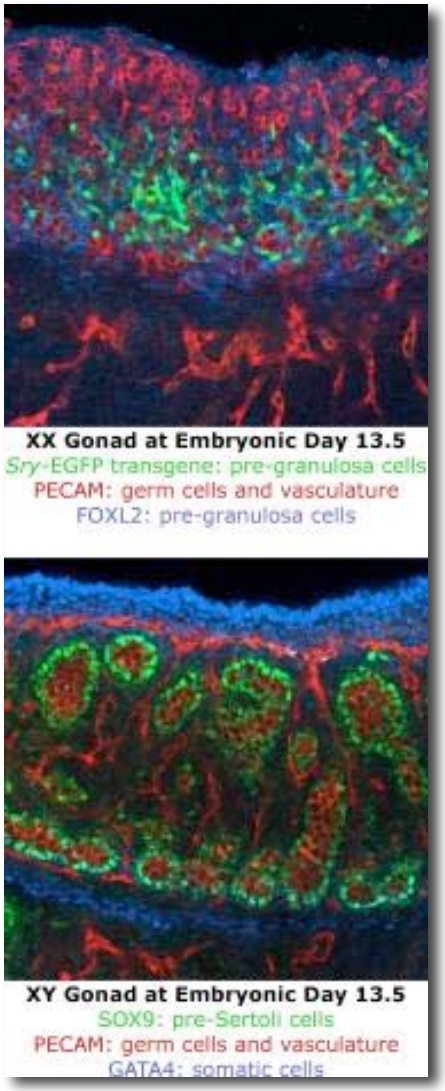
1959 **Welshons & Russell: the role of the Y chromosome**

1991 **Lovell-Badge et al: discovery of the *SRY* gene**

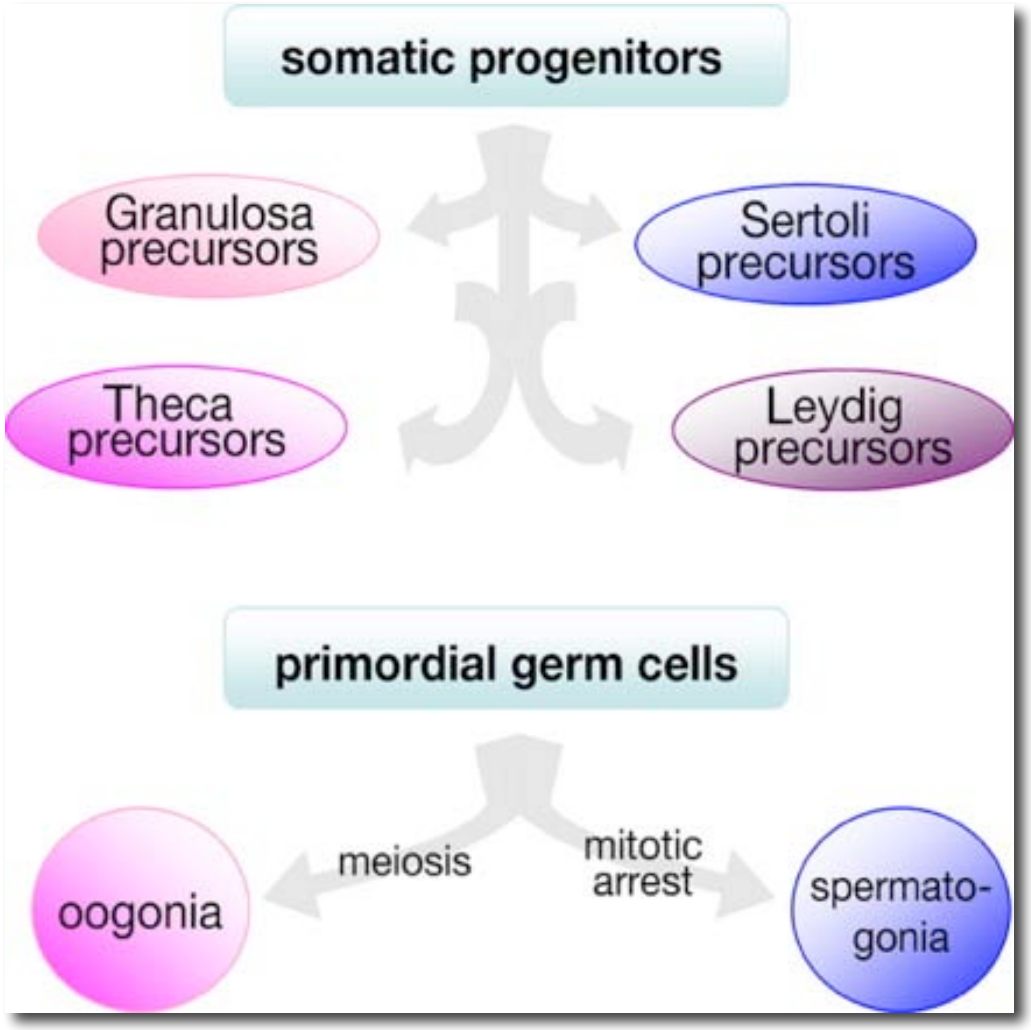




Gonadal Differentiation



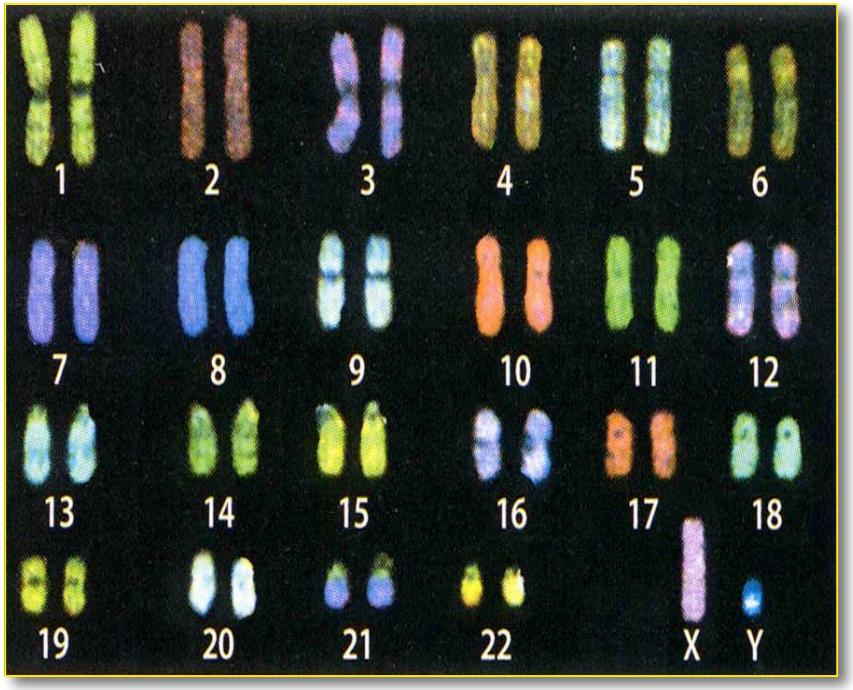
http://gg.bu.edu/people/faculty/albrecht.htm



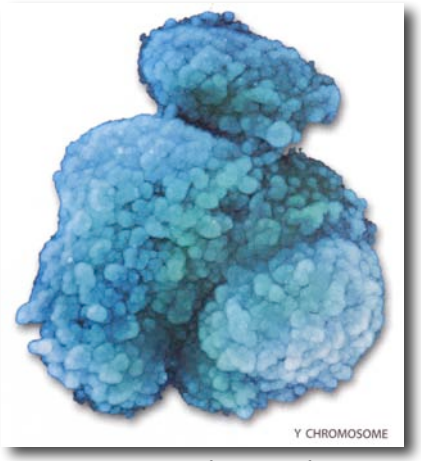
Kim & Capel, Develop Dynamics 235:2292–2300, 2006

Human Primary Sex Determination

- 1. gonadal determination
- 2. chromosomal
  - a. female = XX
  - b. male = XY
- 3. number of X chromosomes not important
- 4. presence of the Y is critical

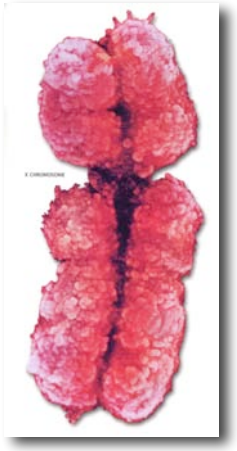


Y Chromosome



- Represents 2% of haploid complement
  - differ between species in size and gene content
  - Contains over 200 genes
  - Contains over 50 million base pairs, of which approximately 50% have been determined
- Genes for
  - Sex determination
  - Histocompatibility
  - Spermatogenesis
  - Growth
  - Cancer

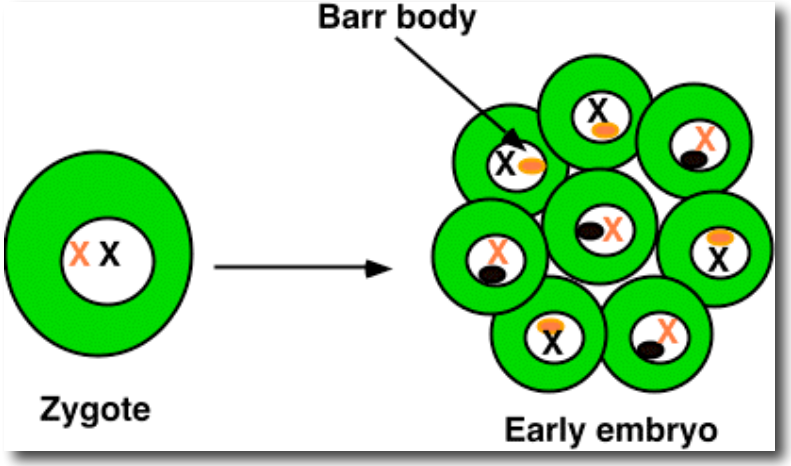
X Chromosome

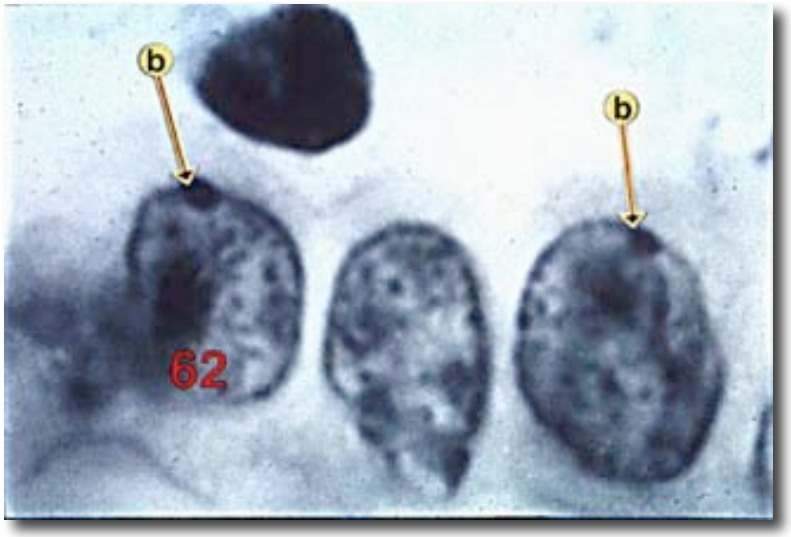


- Contains over 1400 genes
  - ~5% of the haploid genome
- Contains over 150 million base pairs
  - approximately 95% have been determined
- sex linked genes in the X chromosome
  - all these genes will be dominant
    - no opposing genes in the Y chromosome
    - freely expressed in the organisms phenotype
      - hairy ears in old age.
- Sex Linked Characteristics
  - Red-Green color blindness
  - Hemophilia - prevents the clotting of the blood
  - Hairy ears in men through advancing age

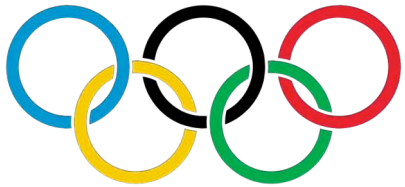
X Inactivation

- Forms the Barr Body
- Condensation of some of the genes on one of the X chromosome
- Why?
  - Double dose of genes thus,
  - Double dose of proteins

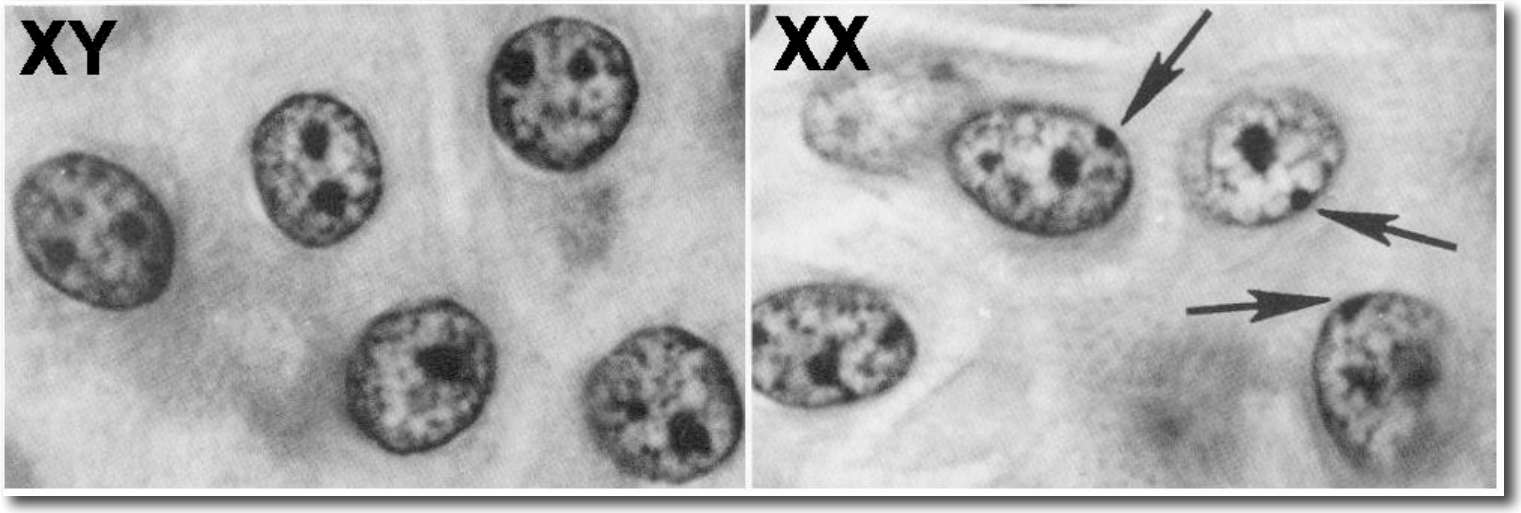




Barr body

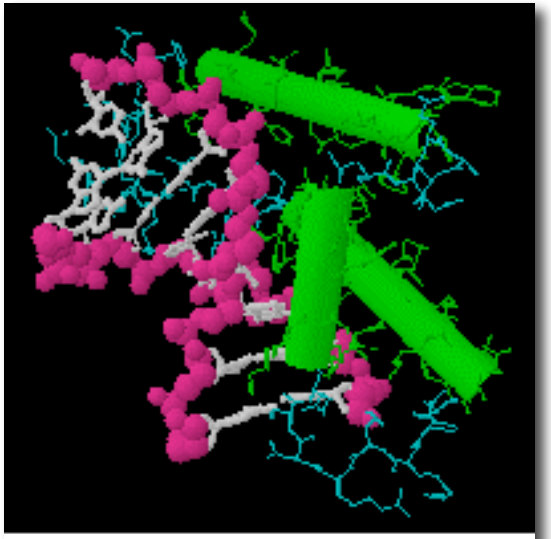


- Mexico City Olympics - 1968
  - introduced genetic testing in the form of a sex chromatin (Barr body)
- Barcelona games
  - PCR for Y chromosome gene, SRY



Sex Determination

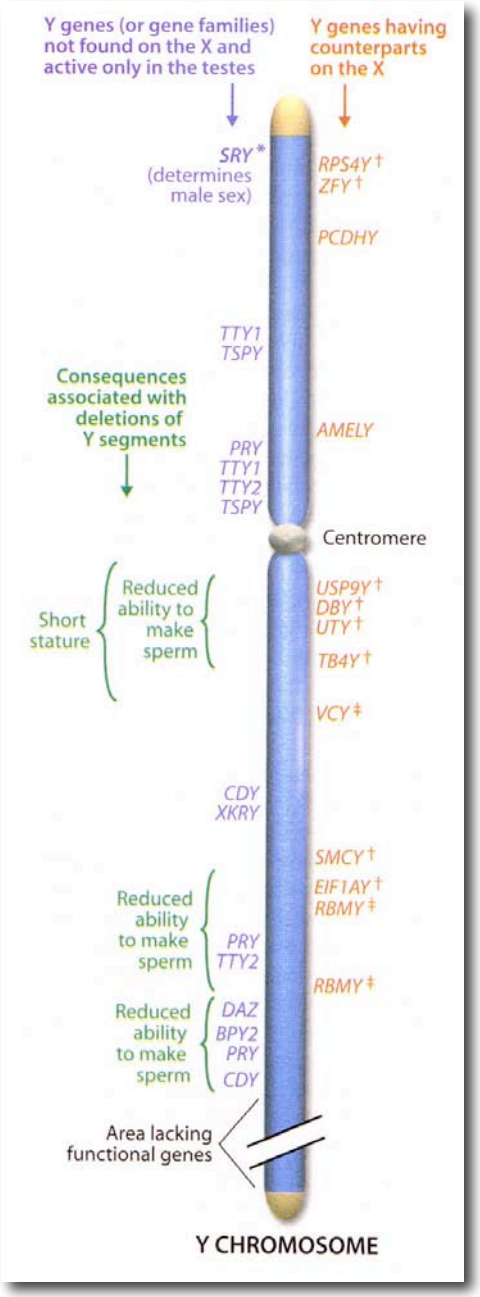
- Transcription factors critical
- Sex determination in mammals
  - Complex - multiple genes
    - SRY, DAX1, SOX9, XH2
    - WT-1 (zinc-finger protein)
    - SF-1 (steroidogenic factor -1)
    - Wnt-4
- SRY critical for testis formation



SRY (green) binds to DNA (pink) and distorts its shape. In so doing, it regulates genes that control the development of the testes.

Chronology

1. 1959: Y chromosome shown to determine males
2. 1966: Testis determining gene localized
   -short arm of Y chromosome
3. 1986-1990: XX males and XY females identified and examined
   - isolated a 35 kilobase in region 1 of Y chromosome
   -the SRY - **S**ex-determining **R**egion of the **Y**



SRY

- A. codes for a 223 AA protein,
- B. a transcription factor
  - ligand unknown
- C. has an HMG box region, found in other transcription factors
  - 'box' binds/folds the DNA
  - essential for sex determination
  - 10-14kb genomic fragment in transgenic mice = sex reversal
  - mutation = sex reversal
- D. found in normal males and XX males (full male genotype)
- E. lacking in normal females and XY females

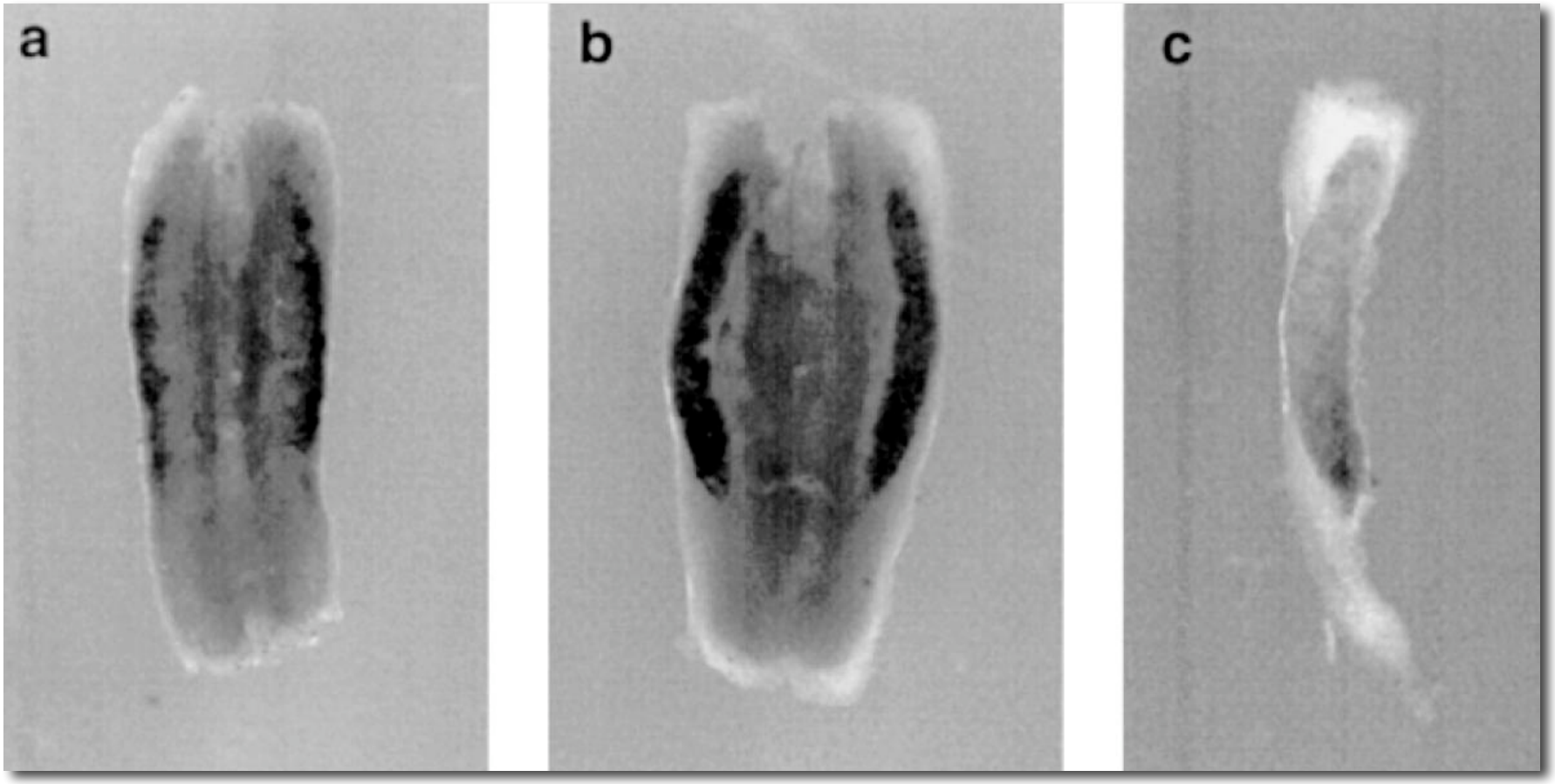
3 Functions of SRY

- 1) differentiation of Sertoli cells
- 2) induces migration of cells from the mesonephros into the genital ridges
- 3) induces proliferation of cells within the genital ridges

Mouse SRY

- homologous region
  - found in developing gonad just before testis formation (2 days prior to testis formation)
  - Also seen in the brain
  - suppressed late in development

Mouse testis formation - in situ whole mount for SRY



Day 14 Day 20 Day 26

Koopman et al J Exp Zool 290 (2001)

Mouse SRY

- inject SRY into XX mouse embryo
  - some develop testis, ducts and penis
    - Gene dosing very important
  - no spermatogenesis
    - normal for XXY males
    - another gene ZFY associated with germ cells
- SRY works with other genes
  - alone does not always give testis
- SRY probably stimulates/blocks a number of genes

TABLE 1. *Genes implicated in sexual development in mammals*

Gene	Protein Function	Gonad Phenotype of Null Mice	Human Syndrome	Reference Nos.
		Bipotential gonad		
Wt1	Transcription factor	Blockage in genital ridge development	Denys-Drash, WAGR, Frasier syndrome	71, 134
Sf1	Nuclear receptor	Blockage in genital ridge development	Embryonic testicular regression syndrome	143, 200, 214
Lhx9	Transcription factor	Blockage in genital ridge development	*	20
Emx2	Transcription factor	Blockage in genital ridge development	*	158
M33	Transcription factor	Gonadal dysgenesis	*	120
		Testis-determining pathway		
Gata4/ Fog2	Transcription/cofactor	Reduced *Sry* levels, XY sex reversal	*	227
Sry	Transcription factor	XY sex reversal	XY sex reversal (LOF); XX sex reversal (GOF)	19, 132
Sox9	Transcription factor	XY sex reversal	Campomelic dysplasia, XX sex reversal (GOF)	10, 21, 48, 76, 230, 241, 242
Sox8	Transcription factor	XY sex reversal in combination with partial loss of *Sox9* function	*	48
Fgf9	Signaling molecule	XY sex reversal	*	51, 206
Dax1	Nuclear receptor	Impaired testis cord formation and spermatogenesis	Hypogonadism	26, 153, 154, 163
Pod1	Transcription factor	XY sex reversal	*	54
Dhh	Signaling molecule	Impaired differentiation of Leydig and PM cells	XY gonadal dysgenesis	23, 43, 44, 49, 189, 237
Pgdra	Receptor	Reduction in mesonephric cell migration	*	31
Pgds	Enzyme	No phenotype	*	1, 145, 245
Arx	Transcription factor	Abnormal testicular differentiation	X-linked lissencephaly with abnormal genitalia	118, 127
Atrx	Helicase	ND	ATRX syndrome	226
Insl3	Signaling factor	Blockage of testicular descent	Cryptorchidism	2, 115, 168, 261
Lgr8	Receptor	Blockage of testicular descent	Cryptorchidism	2, 72, 115
Hoxa10	Transcription factor	Blockage of testicular descent	Cryptorchidism	97, 102
Hoxal1	Transcription factor	Blockage of testicular descent	Cryptorchidism	97, 102
Amh	Hormone	No Müllerian duct degeneration	Persistent Müllerian duct syndrome	14, 15, 100
Misrll	Receptor	No Müllerian duct degeneration	Persistent Müllerian duct syndrome	94, 100
Pax2	Transcription factor	Dysgenesis of mesonephric tubules	*	45
Lim1	Transcription factor	Agenesis of Wolffian and Müllerian ducts	*	128, 129
Dmrt1	Transcription factor	Loss of Sertoli and germ cells	XY female†	194
		Ovary-determining pathway		
Wnt4	Signaling molecule	Müllerian duct agenesis, testosterone synthesis, and coelomic vessel formation	XY female (GOF)	89, 239
FoxL2	Transcription factor	Premature ovarian failure	BPES	53, 175, 207, 236
Dax1	Nuclear receptor	XY sex reversal (GOF)	XY sex reversal (GOF)	110, 163, 223, 257

SOX9

- codes for a transcription factor
  - Protein activates genes in male sex pathway
  - Usually 1 copy of SOX9
- missing copy = COMPOMELIC DYSPLASIA
  - die soon after birth from respiratory distress,
  - Skeletal abnormalities
    - SOX9 induces collagen II gene expression
  - But…75% of XY individuals lacking SOX9 develop as female or hermaphrodites

SOX 9 and Testis

- SOX9 essential for normal testis formation
  - SOX9 only expressed in males on genital ridge
  - co-localized in cells with SRY gene expression
  - not in females

Sry/Sox9/PGs Sertoli Cell Recruitment

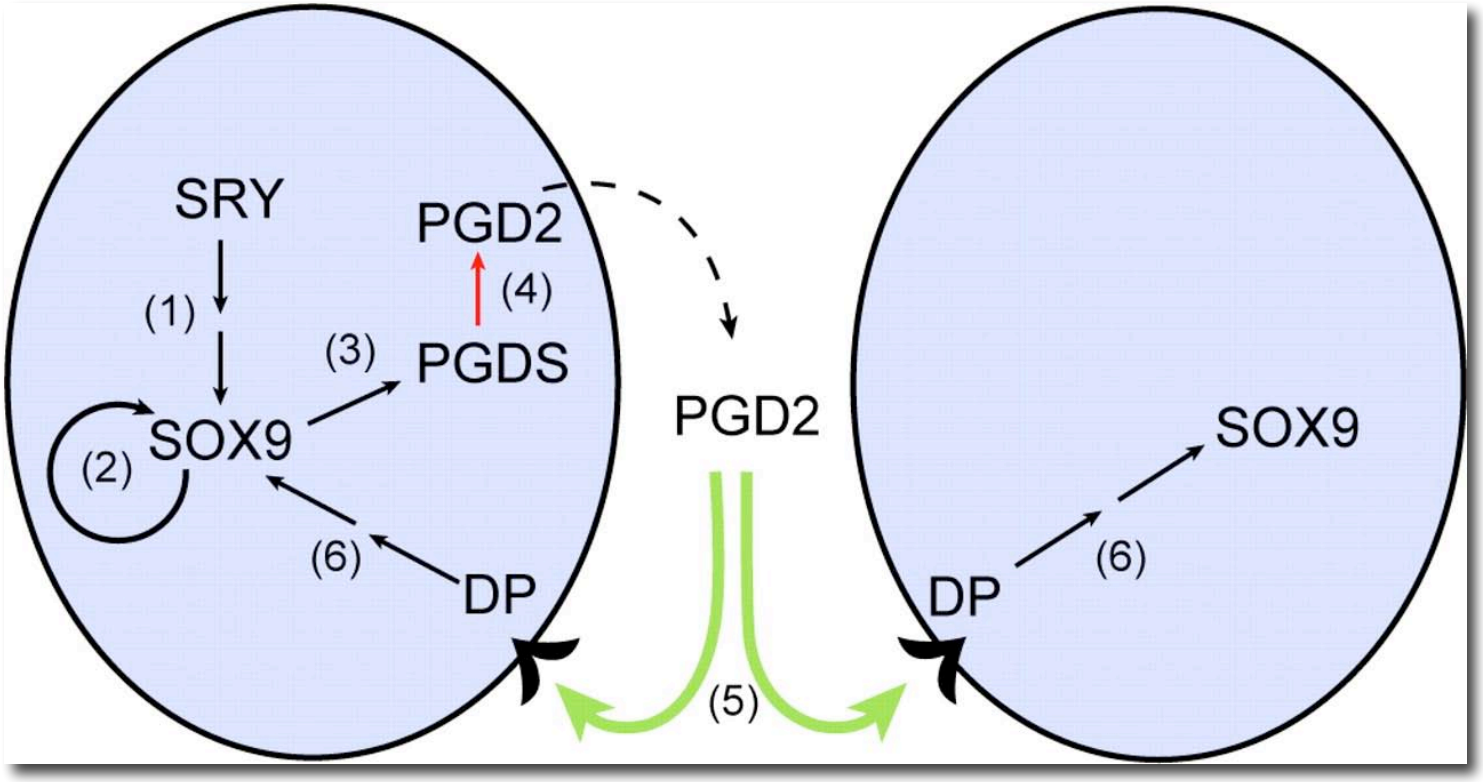


FIG. 8. Model for cell-autonomous and prostaglandin-mediated upregulation of *Sox9* in pre-Sertoli cells. *Sry* induces *Sox9* cell-autonomously either via a direct or indirect regulatory mechanism (*1*). Subsequently, *Sox9* maintains its own expression in an autoregulatory loop (*2*). In addition, *Sry* and/or *Sox9* serve to upregulate *Pgds* (*3*), which leads to prostaglandin D_2 (PGD_2) synthesis (*4*) and secretion. PGD_2 can act by binding to its receptor DP (*5*), to upregulate *Sox9* expression in a paracrine, and possibly also an autocrine manner (*6*). Thus cells that do not express *Sry* or fail to reach a threshold of *Sry* expression can be induced to upregulate *Sox9* and differentiate as Sertoli cells. [Adapted from Smith et al. (216).]

Wilhelm et al. Physiol Rev 87: 1–28, 2007

SF-1 - Orphan Receptor

- a. cofactor with SRY
- b. transcription factor coded for on an autosomal gene
- c. activates genes coding for androgen synthesis
- d. SF-1 present in genital ridge for testis formation, decreases with ovarian development
  - persistence activates
    - MIH in Sertoli cell
    - androgens in Leydig cell
  - Works together with SRY

SF-1 mutation

- lack of SF-1
  - mouse = no gonads or adrenal
  - gonads develop then die
  - animals die due to lack of corticosterone

DMRT-1

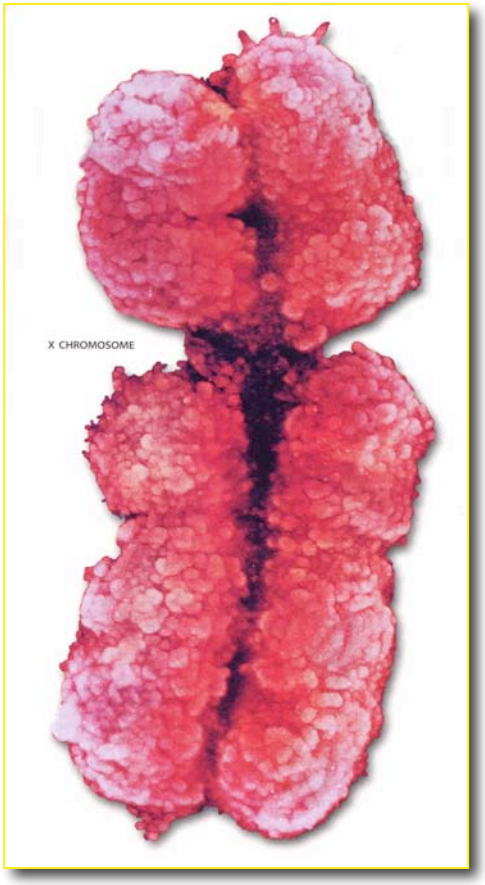
- DM-related Transcription factor
  - Putative sex determining gene in mammal
  - Related to genes determining males in *Drosophilia*
  - Has DNA binding region - DM domain
  - Missing in humans = XY female
  - Expressed in male embryo only (testis)

Other Genes

- XH2
  - X chromosome located
  - helicase family - codes for H type hemoglobin
  - Mutation gives
    - Female phenotype with 46 XY genotype
- DMRT-1
  - Expressed only in male genital ridge
  - Deletion in humans = female phenotype
  - Ortholog DMRT-2 may also be involved
- WT-1
  - Wilms tumor 1 gene
  - Missing/mutation = undifferentiated gonad

Ovarian Development

- Considered default in mammals
- Still requires genetic pathway
- Formed with lack of Y
  - Usually two 'X' chromosomes



Ovarian Development- DAX 1

- 1. potential ovary determining gene
- 2. two sisters - "normal XY" - "Y" was normal
  - duplicated region on the small arm of X (Xp21)
  - two copies - reversed the SRY gene activation
- 3. normal testis formation would override this factor with normal number of DAX1 copies
- 4. codes for a member of nuclear hormone receptor family - gene transcription factors
- 5. Orphan Receptor - ligand unknown
- 6. localized gene activity on the genital ridge

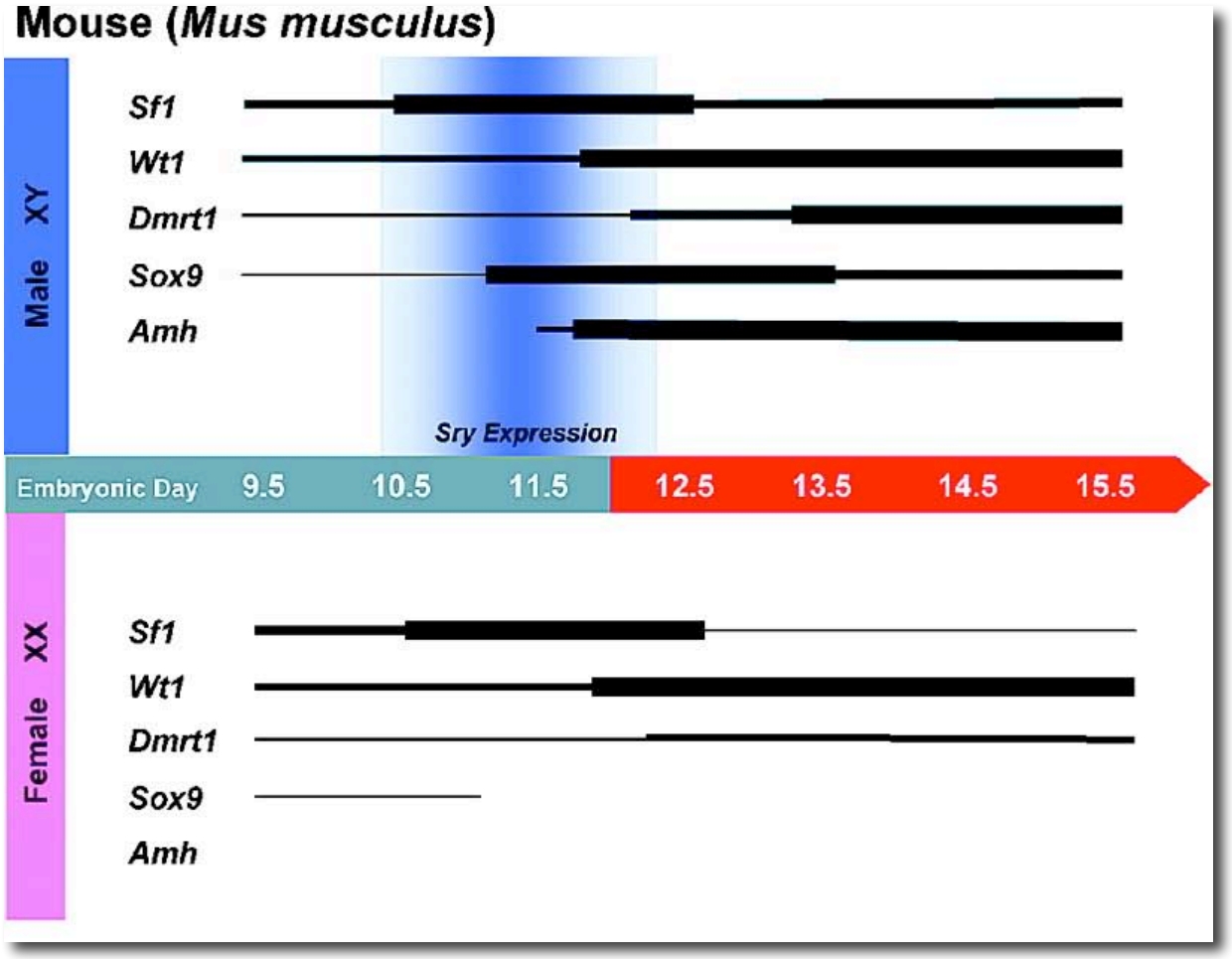
Wnt-4

- 1. Localized gene activity
  - on the genital ridge of mouse
- 2. Expressed in undifferentiated gonad
  - disappears with XY testis formation
  - Absence does not influence testis formation
- 3. with XX genotype
  - ovary forms and Wnt-4 expressed

Missing Wnt-4

- Missing Wnt-4
  - partial female -> male reversal
  - mutant ovary forms
    - Secrete testosterone and AMH (MIH)
    - 3β-HSD and 17α-hydroxylase detected
    - Number of oocytes dramatically reduced
- Similar mutant ovaries seen in αβERKO mice
  - suggested that ER may control Wnt-4
- SRY may repress Wnt4a and activate SF1

Mouse Sex Determination



Yao & Capel J. Biochem. 138, 5–12 (2005)

Fgf9 / Wnt4 Signals

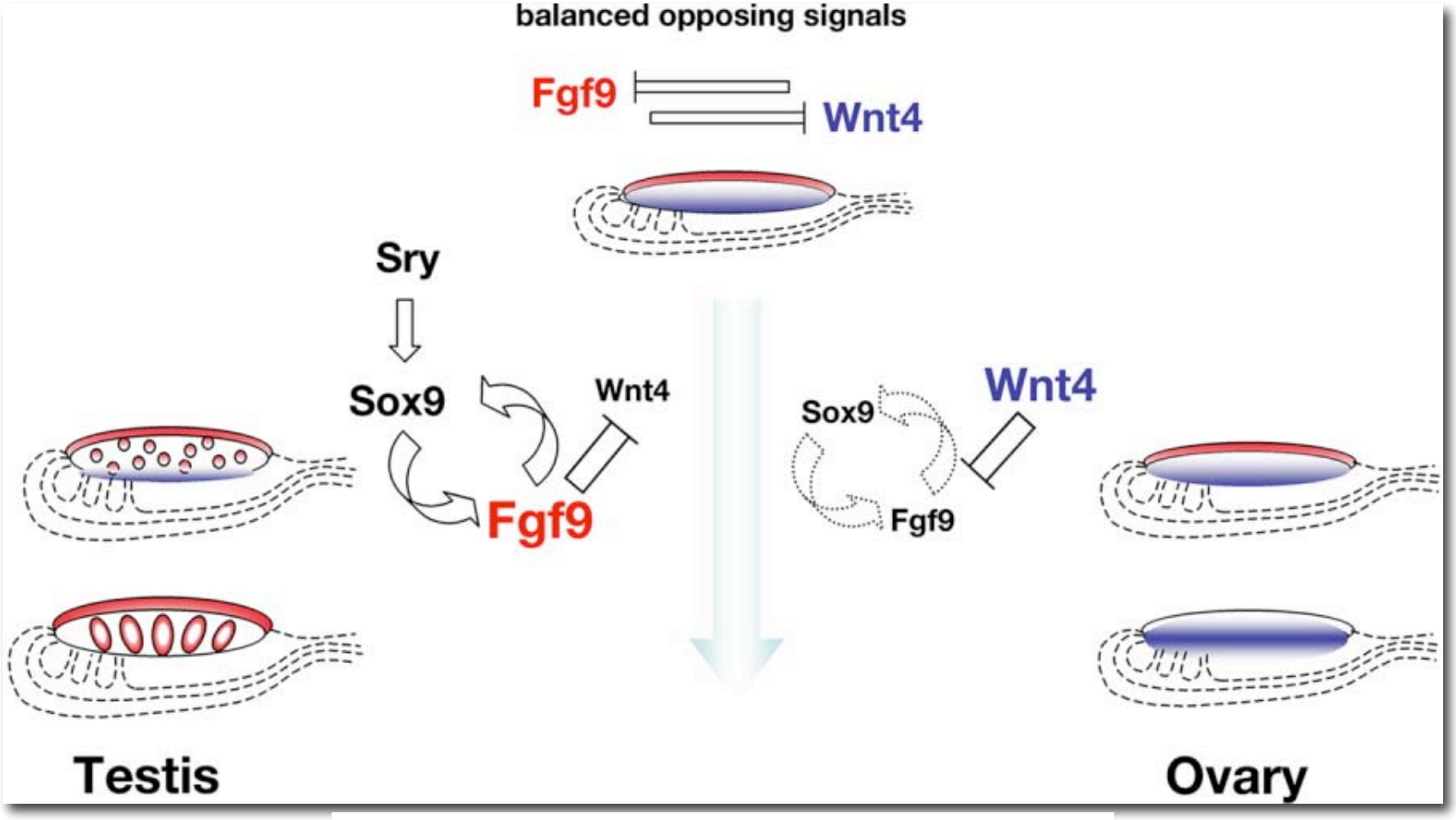
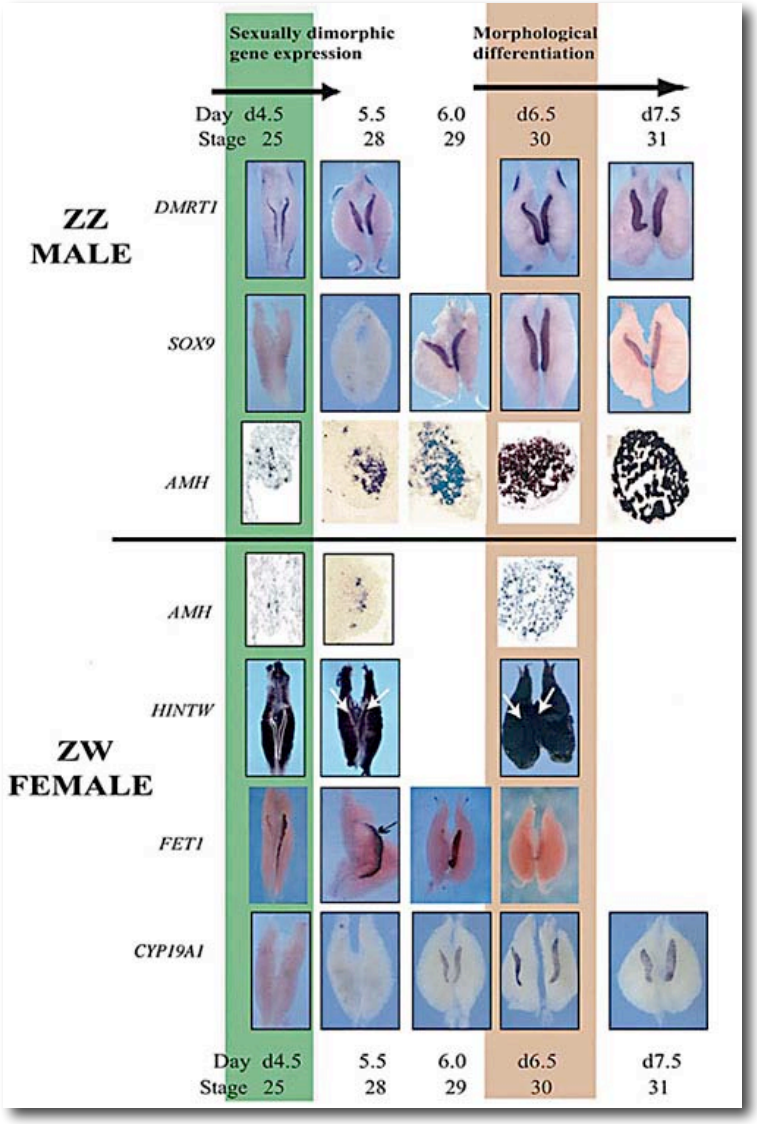


Fig. 3. Model of balanced opposing signals between *Fgf9* and *Wnt4*. In XY gonads, *Sry* upregulates *Sox9* to establish a feed-forward loop that upregulates *Fgf9* and silences *Wnt4*. In XX gonads, *Wnt4* dominates and silences *Fgf9* and *Sox9*.

Kim & Capel, Develop Dynamics 235:2292–2300, 2006

Chicken Gonad Differentiation

Fig. 2. Timing of gene expression in embryonic chicken gonads, as assessed by whole mount and tissue section in situ hybridisation. The onset of morphological differentiation into testes or ovaries is shown (from day 6.5; stage 30). In ZZ males, *DMRT1* mRNA expression is detectable from day 3.5–4.5 (stages 20–25). In comparison, *SOX9* in males is first detectable at day 6.0 (stage 29). In ZW females, *HINTW* mRNA is expressed from days 3.5–4.5 (stages 20–25). *FET1* mRNA is also expressed from days 3.5–4.5, but asymmetrically expressed, with stronger expression in the left gonad. *FET1* expression is down-regulated in the gonads by day 6.5 (stage 30). In comparison, *CYP19A1* is first detectable at day 6.0 (stage 29). *AMH* is first detectable at stage 25 in both sexes, but appears higher in males, according to tissue section in situ hybridisation. By stage 28, this dimorphism in *AMH* is clear (left gonads only are shown). The onset of *AMH* expression precedes *SOX9* expression in males, and *CYP19A1* expression in females. The *AMH* expression is taken from Oreal et al. (1998) with permission.



Smith et al., Cytogenet Genome Res 117:165–173 (2007)

Bipotential Duct System

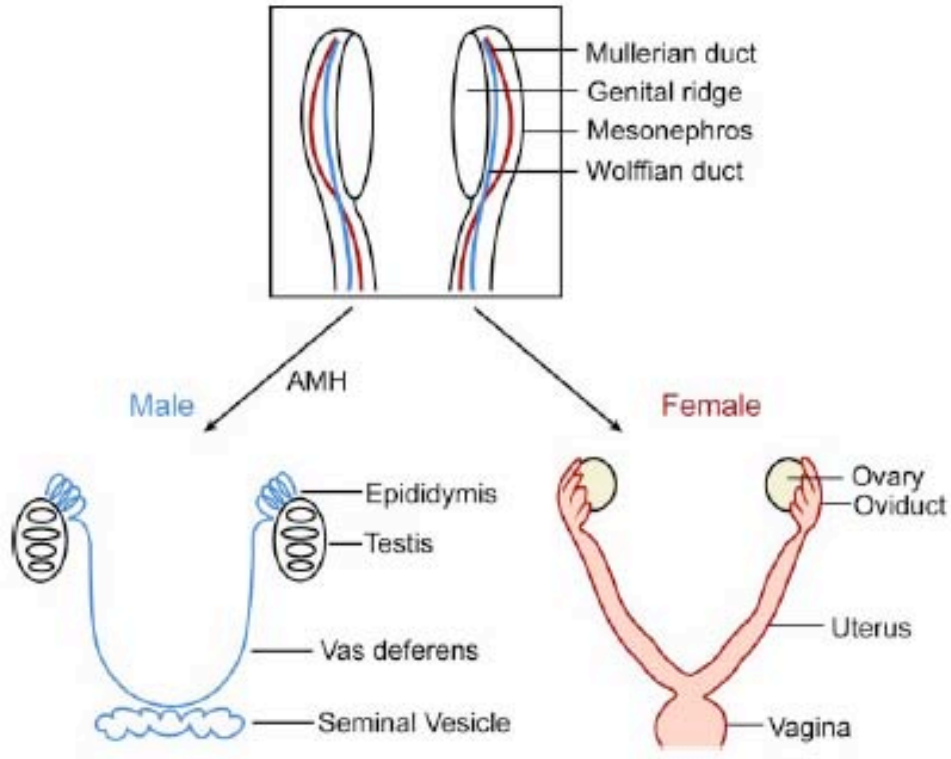


FIG. 4. Development and differentiation of the genital duct system. Both Müllerian and Wolffian ducts are present at the bipotential stage. In males, the Müllerian ducts degenerate under the influence of AMH secreted by the testicular Sertoli cells, whereas the Wolffian ducts differentiate into epididymides, vasa deferentia, and seminal vesicles under the control of androgens produced by Leydig cells. In females, the Wolffian duct regresses and the Müllerian duct differentiates into oviduct, uterus, and upper vagina.

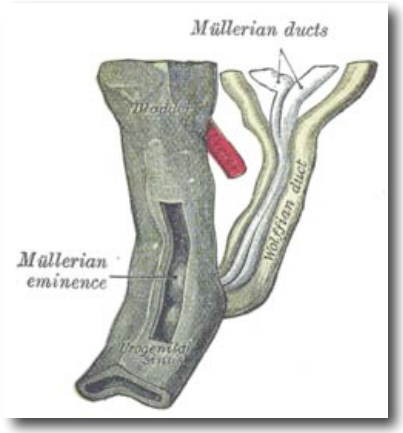
Physiol Rev • VOL 87 • JANUARY 2007 • www.prv.org

Wilhelm et al. Physiol Rev 87: 1–28, 2007

Duct/Genitalia Development

- Hormonal Regulation
- a. MIH (Müllerian Inhibiting Hormone) - Müllerian duct
- b. Androgens
  - 1. ducts - testosterone
  - 2. penis/prostate - dihydrotestosterone

Müllerian Duct Formation



- first description by Johannes Peter Müller in 1830
- origin of the Müllerian duct remains controversial
- lineage-tracing experiments in chicken and mouse embryos
  - show that all Müllerian duct components derive from the coelomic epithelium
  - Müllerian epithelial tube derived from an epithelial anlage at the mesonephros anterior end,
  - segregates from the epithelium and extends caudal of its own accord
    - via a process involving rapid cell proliferation
  - tube is surrounded by mesenchymal cells derived from local delamination of coelomic epithelium
  - no significant influx of cells from the Wolffian duct
  - no support that the tube forms by coelomic epithelium invagination along the mesonephros

Guioli et al. Develop Bio 302:389-398 2007

Bipotential Ducts

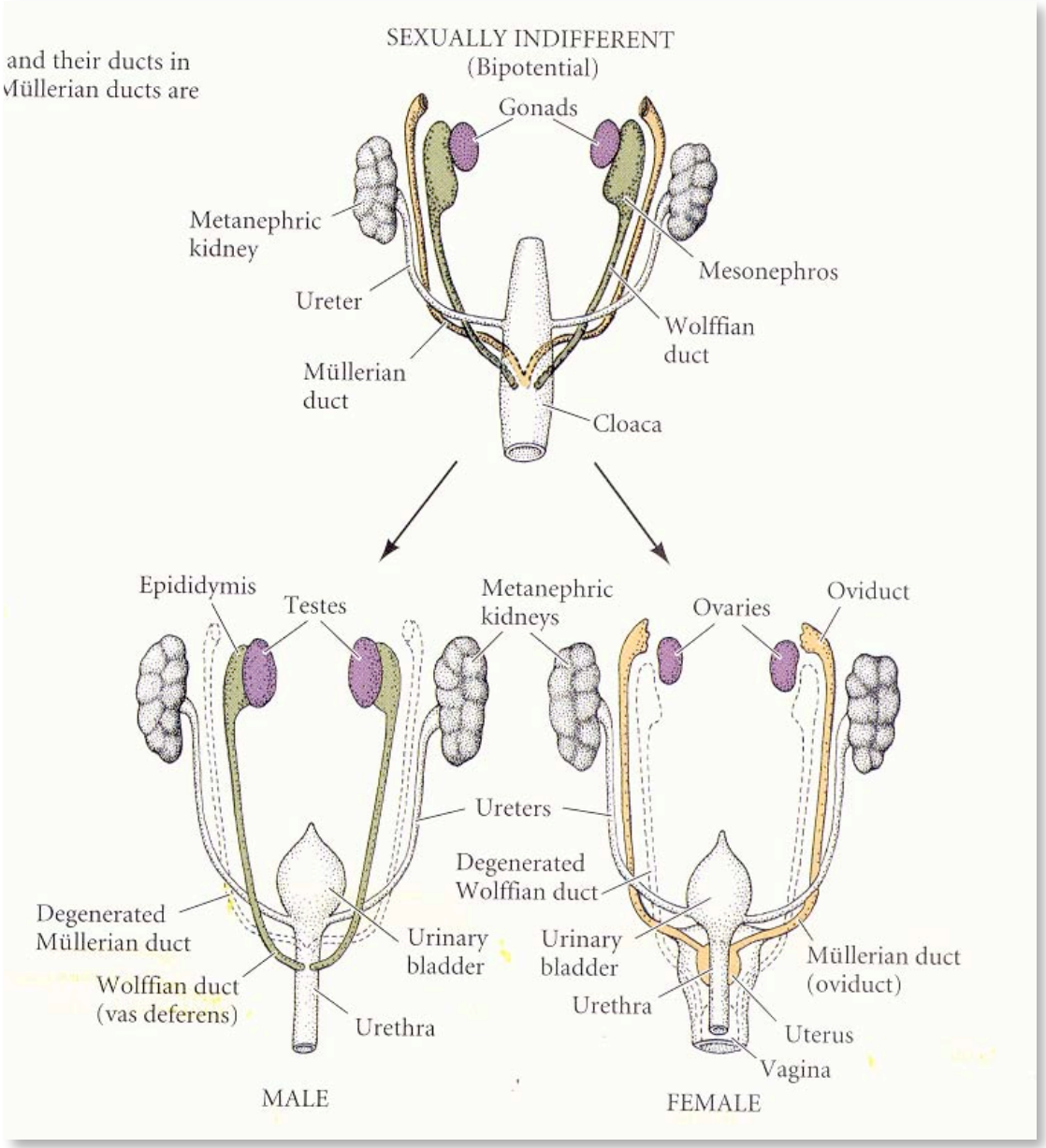


Fig 17.4 Gilbert (2006) Developmental Biology

Secondary Sex Determination

	Male	Female
Wolffian	+ T	- no T
Mullerian	- MIH	+ no MIH

- Body phenotype
  - e.g., duct system
  - usually determined by hormones

a. male = androgens, MIH

b. b. female = no hormones?

Testicular Feminization

Androgen insensitivity syndrome

	Male	Female	TFS
Wolffian	+ T	- no T	? /no
Mullerian	- MIH	+ no MIH	? /no

- XY genetics
- Lack functional androgen receptor
- Testicular formation
- Female external phenotype
- Duct system ?

"Guevodoces"

- "Guevodoces" - "eggs at 12" (Dominican Republic)
  - a. lack functional gene for 5α-reductase 2
  - b. born with blind vaginal sac or poorly fused labia
  - c. at puberty - 12 years - tissue become responsive to testosterone
    - -masculinization of penis, pubic hair - not facial hair
    - -desent of testis into "scrotum"
      - "eggs at 12"- infertile

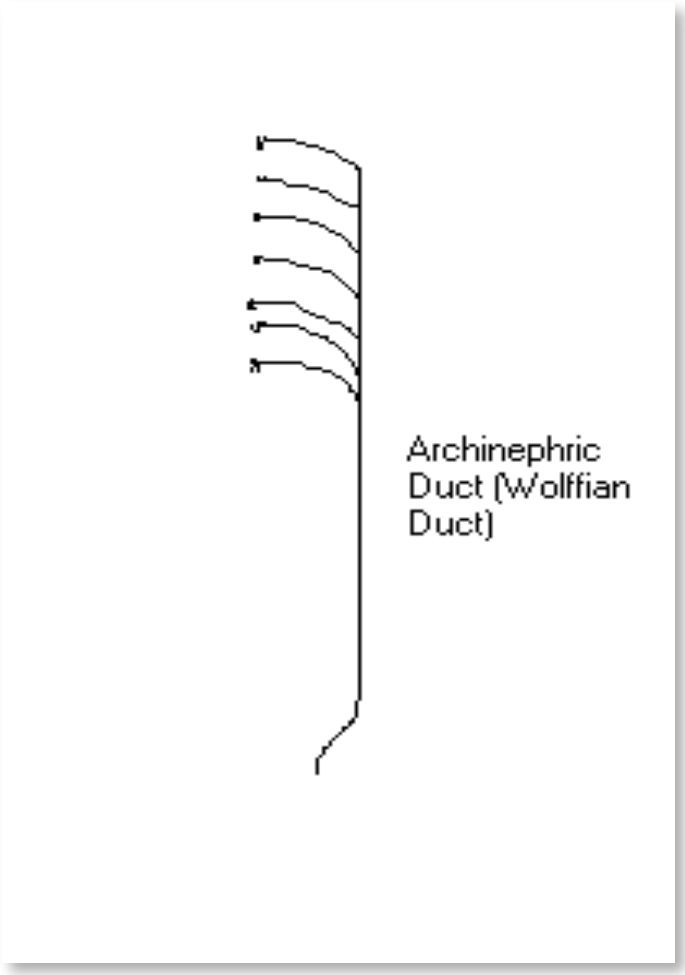
Some web sites

- http://www.pbs.org/wgbh/nova/miracle/determined.html#
- http://herkules.oulu.fi/isbn951426844X/html/i231654.html

Evolution and Embryonic development of the duct system in males

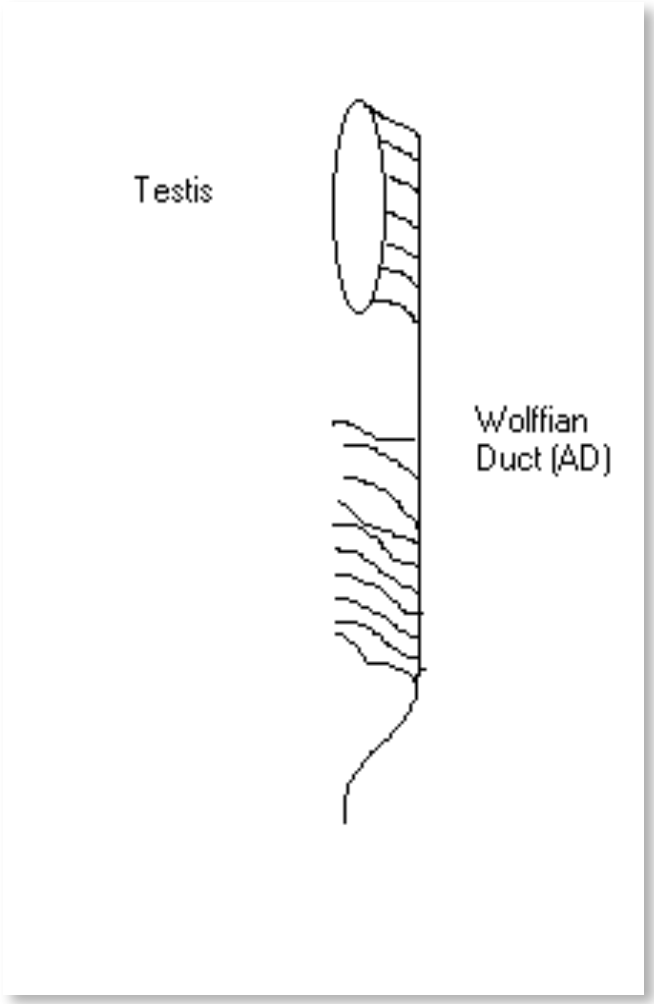
- pronephric kidney
-mesonephric kidney
- metanephric kidney

Pronephric kidney



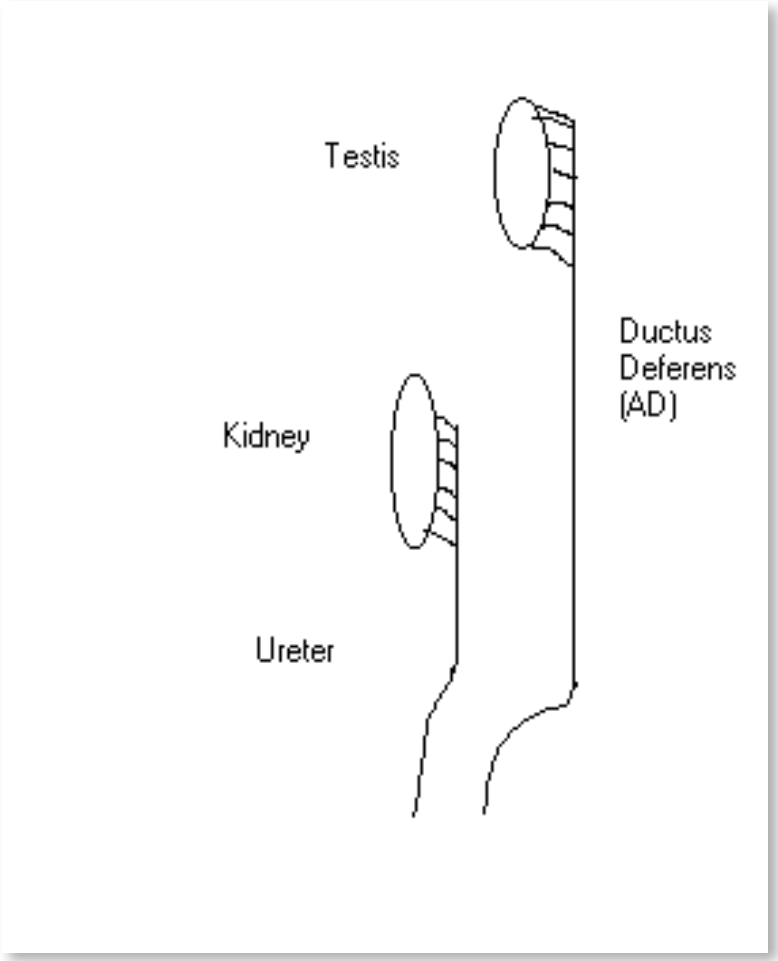
- **1st kidney to form in humans**
  * It is the **functional kidney** of fish and larval amphibians
- Develops anteriorly then degenerates in amniotes
- Remaining duct called the Wolffian Duct (AD)
  - Sperm transport in amniotes

Mesonephric Kidney



- 2nd kidney
- 30 tubules form in humans
- As tubules form caudally the anterior ones die off
- Female mammals- all tubules die
- Male mammals- tubules become sperm ducts of testis
- Functional Kidney: anamniotes

Metanephric kidney (metanephros)



- Permanent kidney of amniotes
- Serves both as an excretory and osmoregulatory organ
- Ureter transports urine
- Ductus Deferens (AD)transports sperm

Proposed Gene Interactions - Testis Differentiation

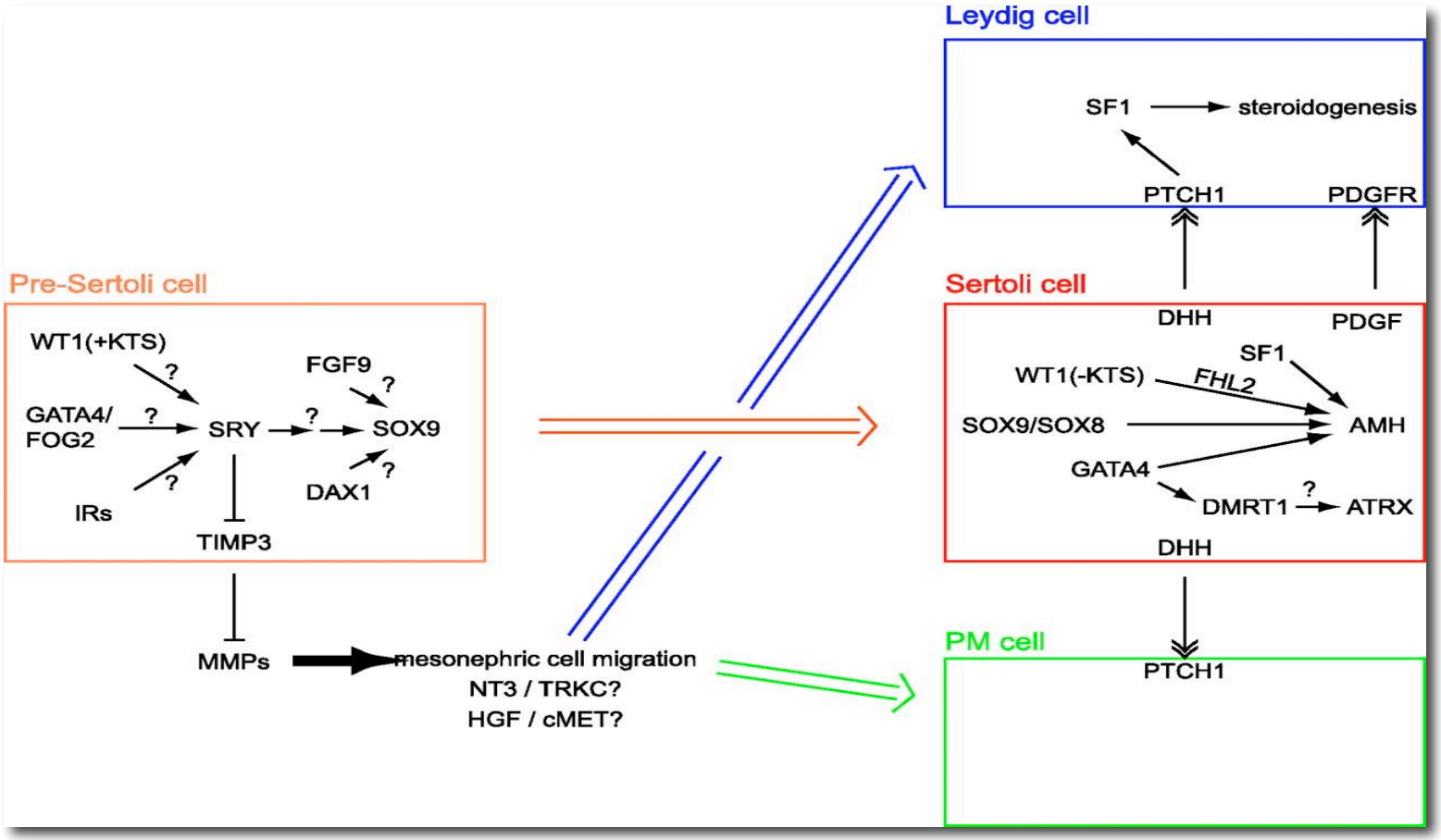
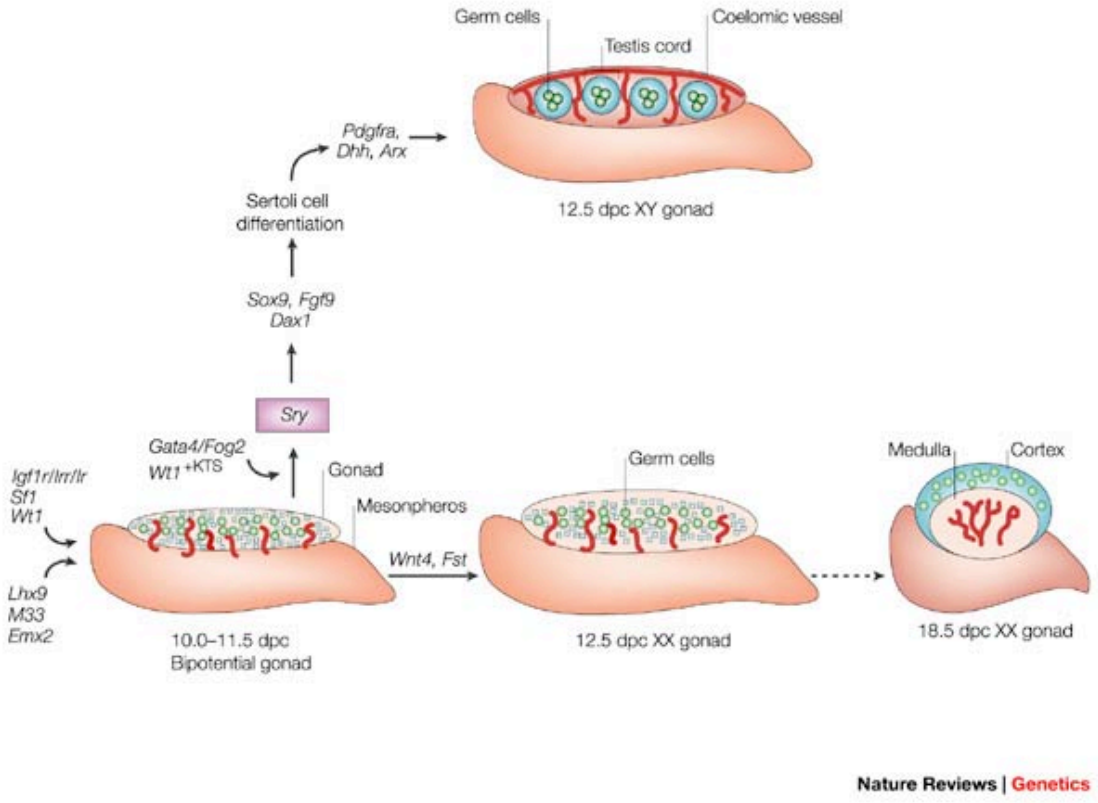


FIG. 13. Postulated interaction of molecular players involved in early testicular development. See text for details. Double-headed arrows, binding to a receptor; colored arrows (blue, red, green), differentiation of precursor cells into testis-specific cell types; black, bold arrow, gene important for cellular process.

Wilhelm et al. Physiol Rev 87: 1–28, 2007

Bipotential Gonad

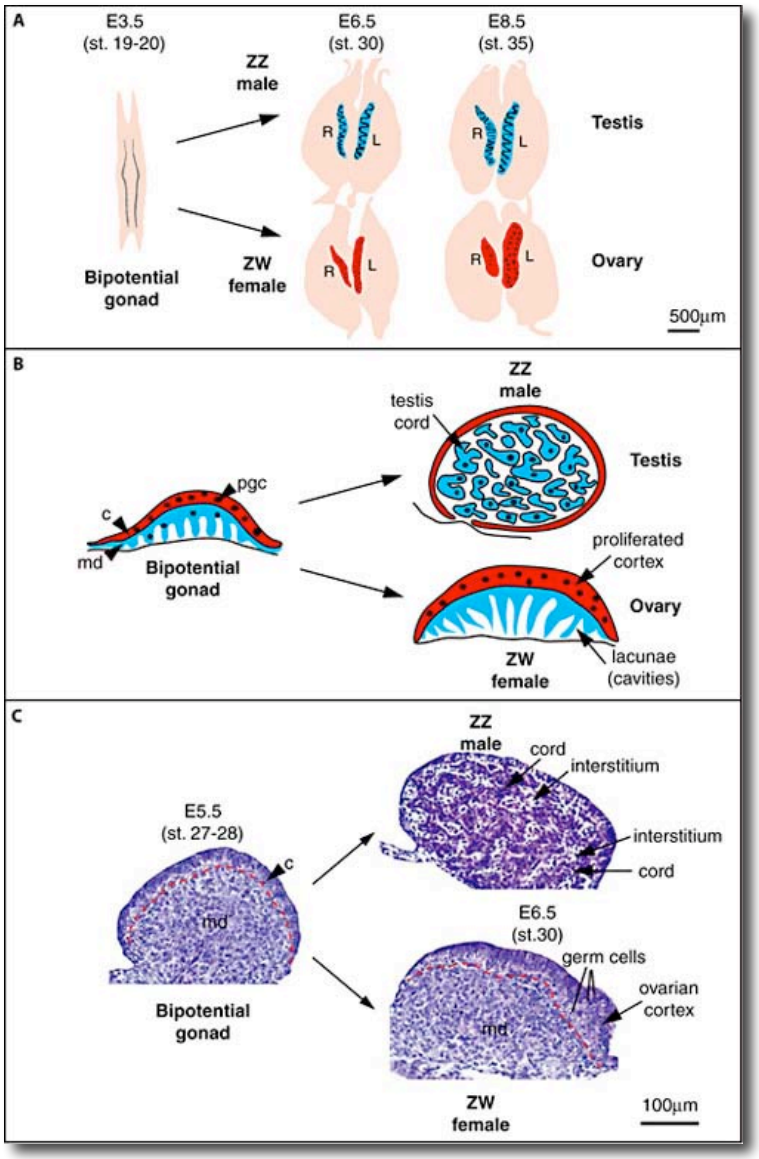


- Several factors are required between 10.5–11.5 days post coitum (dpc) for the outgrowth of the early bipotential gonad by preventing apoptosis or promoting cell proliferation (Sf1, Wt1, Lhx9, M33, Emx2, Igf1r/Ir/Irr). Between 10.5–12.0 dpc, GATA4/FOG2 and WT1+KTS are implicated in the activation of Sry expression in the XY gonad. Sry expression diverts the XY gonad towards the testis fate. Sox9, Fgf9 and Dax1 are implicated in the early steps of the male pathway after the initiation of Sry expression. Downstream signalling pathways promote the rapid structural changes that characterize early testis development (Pdgf, Dhh, Arx). By contrast, few morphological changes are apparent in the XX gonad until near birth (18.5 dpc), when ovarian follicles begin to form in the ovarian cortex. Wnt4 and Fst are the only two genes with characterized functions in early ovarian development. Arx, aristaless related homeobox; Dax1, nuclear receptor subfamily 0, B1 (Nr0b1); Dhh, desert hedgehog; Emx2, empty spiracles homologue 2; Fgf9, fibroblast growth factor 9; Fog2, zinc finger protein, multitype 2 (Zfpm2); Fst, follistatin; Gata4, GATA binding protein 4; Igf1r, insulin-like growth factor 1 receptor; Ir, insulin receptor; Irr, insulin receptor-related receptor; Lhx9, LIM homeobox protein 9; M33, chromobox homologue 2 (Cbx2); Pdgf, platelet-derived growth factor; Sf1, nuclear receptor subfamily 5, group A member 1 (Nr5a1); Sox9, Sry-like HMG-box protein 9; Wnt4, wingless-related MMTV integration site 4; Wt1, Wilms tumour homologue.

Brennan & Capel Nature Reviews Genetics 5, 509-521 (July 2004)

Bipotential Gonad

Fig. 1. Gonadal development and sexual differentiation in the chicken embryo. (**A**) Schematic of gonadal anatomy. At embryonic day 3.5 (stage 19–20), the gonads are undifferentiated or bipotential (shown in blue), on the medial surface of the mesonephric kidneys (pale brown). In ZZ males, bilateral testes develop, while, in ZW females, the left gonad becomes an ovary and the right regresses. (**B**) Schematic of gonadal histology. The bipotential gonad comprises an outer cortical layer (c), and underlying medulla (md). Primordial germ cells (pgc) are concentrated mainly in the cortex. Testis formation involves the condensation of medullary cords into seminiferous (testis) cords. The (left) ovary is characterised by cortical proliferation, while the medulla becomes reticulated, with numerous cavities (lacunae). (**C**) Gonadal histology in the chicken embryo. At embryonic day 5.5 (E5.5; stage 27–28) the gonads are histologically undifferentiated. The cortex (c) is distinct from the underlying medulla (md). In ZZ males, condensing cords are apparent by E6.5 (stage 30). The interstitium (site of Leydig cell development and testosterone synthesis) is present between the cords. In contrast, cortex proliferation, including germ cell proliferation, is apparent in ZW females.



Smith et al., Cytogenet Genome Res 117:165–173 (2007)