Sex Determination

1. Genotypic sex determination

<table>
<thead>
<tr>
<th>Species</th>
<th>Mechanism</th>
<th>Sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musca domestica</td>
<td>CSD: dominant Y</td>
<td>· XX female</td>
</tr>
<tr>
<td>Birds</td>
<td>CSD: ratio (T)</td>
<td>· ZZ female</td>
</tr>
<tr>
<td>Turtles</td>
<td>ESD: temperature</td>
<td>· warm = female</td>
</tr>
<tr>
<td>Drosophila</td>
<td>CSD: X/A ratio</td>
<td>· XX female</td>
</tr>
<tr>
<td>Musca domestica</td>
<td>CSD: dominant M locus</td>
<td>· m/m female</td>
</tr>
<tr>
<td>Apis mellifera</td>
<td>CSD: haplo-diploidy</td>
<td>· diploid female</td>
</tr>
<tr>
<td>Nematodes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caenorhabditis</td>
<td>CSD: X/A ratio</td>
<td>· XX female</td>
</tr>
<tr>
<td>Meloidogyne</td>
<td>ESD: population density</td>
<td>· sparse = female</td>
</tr>
</tbody>
</table>

2. Environmental sex determination

- Turtles use temperature
  - > 32°C produces females
  - < 28°C produces males

- CSD = chromosomal sex determination, ESD = environmental sex determination
CHROMOSOMAL SEX DETERMINATION IN MAMMALS

- MAMMALS - the presence of either a second X chromosome or a Y chromosome determines whether the embryo is female (XX) or male (XY)
- FLIES - the ratio of X chromosomes to autosomes determines sexual phenotype.

PRIMARY AND SECONDARY SEX DETERMINATION IN MAMMALS

- PRIMARY SEX DETERMINATION:
  1. Establishment of chromosomal sex at fertilization
  2. The development of the undifferentiated gonads into testis or ovaries

- SECONDARY SEX DETERMINATION:
  The sequential differentiation of internal and external genitalia in accordance with gonadal sex (phenotypic sex).

1. ESTABLISHMENT OF CHROMOSOMAL SEX AT FERTILIZATION

The Y chromosome carries the testis determining factor. This factor will organize the gonad into a testis rather than an ovary. The mammalian Y chromosome is a crucial factor for determining sex in mammals.

Evidence for the Y chromosome mechanism:
1. Y chromosome confers maleness and determines sex.
2. Verified by studies of non-disjunction aneuploidy:

<table>
<thead>
<tr>
<th>Aneuploidy</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>XO* Turner Syndrome*</td>
<td>Female</td>
<td>1:10,000</td>
</tr>
<tr>
<td>XY</td>
<td>Male</td>
<td>1:1000</td>
</tr>
<tr>
<td>XXX</td>
<td>Female</td>
<td>Normal though sometimes less fertile</td>
</tr>
</tbody>
</table>

2. DEVELOPMENT OF THE GONAD

The indifferent gonad develops in the context of the larger urogenital system. Three overlapping, sequential kidney systems: the pronephros, the mesonephros, and the metanephros. The three regions are further divided: the metanephric mesenchyme and the ureteric bud.

The kidney is a product of the interaction between the metanephric mesenchyme and the ureteric bud.
A second duct, the Mullerian duct, arises within the mesonephros by invagination of the coelomic epithelium. This duct runs parallel to the Wolfian duct but turns toward the midline at the posterior end of the mesonephros. The gonads arise from a thickening in the ventrolateral surface of each mesonephros. During this indifferent stage, the genital ridge epithelium proliferates and extends into the loose connective mesenchymal tissue above it. These epithelial layers form the SEX CORDS. The germ cells migrate into the gonad and are surrounded by the sex cords.

**TESTIS DEVELOPMENT**

If the fetus is XY, the sex cords continue to proliferate extending deeply into the connective tissue. These cords form a network of internal sex cords and at the most distal end, the thinner rete testes. Eventually the cord, now called testis cords, lose contact with the surface epithelium and become separated from it by a thick extracellular matrix, the tunica albuginea. When germ cells enter the male gonads, they will develop in these testis cords.

**OVARIAN DEVELOPMENT**

In females, the germ cells will reside near the outer surface of the gonad. Unlike the sex cords in males which continue their proliferation, the initial sex cords of XX sex cords degenerate. However, the epithelium soon produces a new set of sex cords which remain near the outer surface (Cortex) of the organ, (CORTICAL SEX CORDS). These cords are split into clusters, with each cluster surrounding a germ cell. The germ cells will become ova, and the surrounding sex cord will differentiate into granulosa cells. The mesenchymal cells of the cortex and medulla will differentiate into the theca cells and form follicles that envelope the germ cells and secrete steroid hormones. Each follicle will contain a germ cell that enters meiosis. In females the Mullerian duct remains intact and differentiates into the oviducts, uterus, cervix and upper vagina.

**SUMMARY OF THE DEVELOPMENT OF THE GONADS AND THEIR DUCTS IN MAMMALS**

The testis cord will differentiate into Sertoli cells which secrete anti-Mullerian hormone and later support the development of sperm. At puberty the cords hollow out to form the seminiferous tubules and the germ cells migrate to the periphery and differentiate. In the mature seminiferous tubule, the sperms are transported from inside the tube through the rete testis. These tubules will link the testis to the Wolfian duct which differentiates into the epididymis and vas deferens, the tube through which the sperms pass into the urethra. During development interstitial mesenchymal cells differentiate into Leydig cells which make testosterone.

**THE CELLULAR COMPONENTS OF THE "INDIFFERENT" Gonad**

- Testis
- Genital Ridge
- Ovary
- Prospereomatozoids
- Primordial Germ cells
- Oocytes
- Sertoli cells
- Supporting Precursors
- Steroid Cell Precursors
- Follicle Cells
- Leydig Cells
- Steroid Cell Precursors
- Intestinal (Theca) Cells
- Tunica Peribularadial Myoid Cells
- Tunica (Theca) cells

**WHAT IS THE SITE OF Y CHROMOSOME (TESTIS DETERMINING FACTOR) ACTION?**
• GERM CELLS: Testis develop normally in embryos homozygous for the mutation at the W (or white spotting) locus, which compromises the ability of germ cells to proliferate and colonise the genital ridges. The germ cells are therefore irrelevant to testis determination.

• Of the three somatic cell lineages the “supporting” cell appears to be the most critical for testis determination. Burgoyne and colleagues looked at the sex chromosome constitution of cells within testes of XX-XY chimeras, and found that Sertoli cells are almost exclusively XY, whereas the proportion of XX and XY cells in other gonadal lineages was the same as that found elsewhere in the embryo. This suggests that the testis determining gene acts cell autonomously within the supporting cell precursor.}

WHAT IS THE SITE OF Y CHROMOSOME (TESTIS DETERMINING FACTOR) ACTION?

Chimeric mice made from XX and XY cells develop into males if ≥ 30% XY cells.

In the testis, only Sertoli cells show a strong bias for the Y chromosome. Palmer and Burgoyne, 1991

SERTOLI CELLS ARE THE SITE OF Y CHROMOSOME (TESTIS DETERMINING FACTOR) ACTION

WHAT IS THE SITE OF Y CHROMOSOME (TESTIS DETERMINING FACTOR) ACTION?

Palmer and Burgoyne, 1991

THE MECHANISMS OF MAMMALIAN PRIMARY SEX DETERMINATION

Humans have provided the most direct route to cloning the gene responsible for sex determination.

1. The testis determining factor lies close to the XY pairing region, so that sex reversal through abnormal XY interchange is relatively common in humans.

2. Mutations in sex determining genes do not result in an immediately noticeable phenotype - the result is a gender that does not correspond to normal sex chromosome constitution. Thus it has been possible to collect DNA from many patients with abnormalities in sex determining genes.

SRY: THE Y CHROMOSOME SEX DETERMINANT

By analyzing the DNA of XX men and XY women, the position of the testis determining gene was narrowed down to a 35,000 bp region of the Y chromosome located near the tip of the short arm (Sinclair et al., Nature, 1991)
Characteristics of SRY protein:
- Codes for a 223 a.a. protein
- A transcription factor
- HMG DNA binding domain:
  - ‘box’ binds DNA in a sequence specific manner (AACAAT)
  - HMG domain binds DNA in the minor groove, induces a dramatic bend in the helix may function as an architectural component of chromatin.

**DIRECT EVIDENCE THAT SRY IS THE SEX DETERMINING GENE**

- **1. MUTATION STUDIES:** Evidence that mutations within SRY disrupt the function of sex determination has been accumulated both in humans and mice. In a study of sex reversed humans who carry a Y chromosome, test positive for SRY but develop as females, ~10-15% possess mutations specifically within the DNA binding domain of SRY. Some of these are small deletions while others involve point mutations leading to a.a. substitutions or frame shift.

- **2. TRANSGENIC STUDIES:** Transgenic experiments have demonstrated that Sry is the only gene from the Y chromosome necessary for testis determination.


  - XX mouse transgenic for Sry is male. A. PCR shows presence of the Sry gene in normal XY males and in transgenic Sry mouse. The gene is absent in the female XX littermate.
  - B. The external genitalia of transgenic mouse are male and essentially the same as in an XY male.

- **3. EXPRESSION STUDIES:** In the developing mouse embryo, Sry is expressed in the male genital ridge during the window of development when the gonad condenses and testis cord formation is initiated.
1. MUTATION STUDIES: Transgenic experiments have demonstrated that Sry is the only gene from the Y chromosome necessary for testis determination.

2. TRANSGENIC STUDIES: In the developing mouse embryo, Sry is expressed in the male genital ridge during the window of development when the gonad condenses and testis cord formation is initiated.

3. EXPRESSION STUDIES: Chimeric studies derived from XX and XY cells have been used to determine the cell lineages involved in sex determination. Palmer and Burgoyne, 1992 repeat these experiments with SRY- and SRY+ cells.

4. CHIMERIC STUDIES: Chimeric experiments have demonstrated that Sry is the only gene from the Y chromosome necessary for testis determination.

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FUNCTIONS OF SRY

1. Differentiation of Sertoli cells?
2. Induces migration of cells from the mesonephros into the genital ridges?

Capel et al., have shown that Sry works via an indirect mechanism. Sry in the genital ridge cells induces these cells to secrete a chemotactic factor that permits migration of mesonephric cells into the XY gonads. These mesonephric cells then induce the genital ridge cells to become Sertoli cells with male-specific gene expression patterns.

HOW WAS THE EXPT DONE? Specifically, when XX gonads with either XX or XY mesonephros, the mesonephric cells did not enter the gonads. However, when XX or XY mesonephros is cultured with XY gonads, the mesonephric cells enter the gonad. There was a strict correlation between the presence of Sry in the gonadal cells, mesonephric cell migration, and formation of testis cords. SRY may function at least in part, indirectly to create tissue by inducing mesonephric cell migration into the gonad.

FGF9 is a candidate secreted factor to regulate migration of cells from the mesonephros into the genital ridges:

Gonadal rudiments for XX mice could induce mesonephric cell migration. These rudiments were isolated with FGF9.

Next expt: what happens in FGF9 targeted disruption

FGF9-/- are female
SOX9: an autosomal testis-determining gene

What functions to induce Sertoli cell differentiation? Transient nature of Sry expression in the gonad suggests that it is not involved in the maintenance of cell identity or cell function. Therefore, SRY must activate other genes that are involved in defining and maintaining Sertoli cell identity.

SOX9- (Goodfellow group)

Individuals having only one functional copy of SOX9 have a syndrome called Campomelic Dysplasia, a disease involving numerous skeletal and organ systems. About 75% of XY patients with this syndrome develop as phenotypic females or hermaphrodites.

XX humans who have an extra copy of SOX9 develop as males, and XX mice made transgenic for Sox9 develop testes.

SF1: the link between SRY and male developmental pathways

SF1- (Steriodogenic factor)

- expressed in Leydig and Sertoli cells
- directly interacts with SOX9 to elevate AMH transcription
- in humans, SF1 mutations have malformed fibrous gonads and retain fully developed Mullerian duct structures.

DEVELOPMENT OF THE GONAD

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SUMMARY OF THE DEVELOPMENT OF THE GONADS AND THEIR DUCTS IN MAMMALS


DAX1: a potential testis-suppressing gene on the X chromosome

- Encodes a member of the orphan nuclear hormone receptor family
- C-terminal is homologous to ligand-binding domain
- N-terminal 3.5 repeats with zinc-finger domains (DBD binding domain)
- Encodes a member of the nuclear hormone receptor family, expressed in the genital ridges of both male and female mouse embryos, and is seen in male mice shortly after Sry expression. Xy mice, Sry and Dax1 are expressed in the same cells.
Dosage-sensitive sex reversal: an 'anti-testis' role for DAX1

Patients with DSS have a XY karyotype and no testes

Swain et al., generated a mouse model to determine whether Dax1 was responsible for DSS. Transgenic mice carrying extra copies of Dax1 were bred. Sex reversal was not observed, however the mice with highest levels of Dax1 showed retarded testes formation.

DAX/DAX

+/-       XX       XX       XY       XY

Dax1-/- mice are XY sex reversed, they neither have testicular cords, Sertoli or Leydig markers

A window of activity, the action of DAX1 in male sex determination:

For testes formation to ensue, both positive and negative regulatory processes involving DAX1 are required. If DAX1 is raised (by gene duplication) or reduced (by mutation) abnormalities of testis formation occur.

WNT-4 REGULATES FEMALE DEVELOPMENT

WNT-4 expression:

Before gonadal formation (9.5-10.5 mouse) it is expressed along the length of the mesonephros in the mesenchyme.

WNT-4 is expressed in the mesenchyme of the indifferent gonad in both sexes but is absent from the Wolffian duct.

When sex specific differentiation of the gonads commences (11.5) WNT-4 is downregulated in the male gonad but maintained in the female, specifically in the mesenchymal cells of the Mullerian duct.

Masculinization of Wnt-4 mutant females

Neither male or female WNT-4 -/- initially develop a Mullerian duct, however only in females that the absence of the Mullerian duct has functional consequences.

Summary of possible mechanism for primary sex determination in mammals