Sex determination and differentiation
Outline

- Sexual phenotype
- Sex determination *versus* sex differentiation
- Sex determination
  - sex-determining genes
  - dosage compensation
- *Drosophila* sex determination
- Mammalian sex determination
- Sex differentiation → werkcollege
Most organisms have two sexes

Sexes are defined by:

- Mutual incompatibility between the same mating type
- Anisogamy (*i.e.* the production of large and small gametes)

**Female gametes (eggs):**
- Few, large, immobile,
  - include resources

**Male gametes (sperm):**
- Numerous, small, motile

- **Monoecious species (hermaphrodites):**
  - Some animals (snail, earthworms...), most plants

- **Diecious species (separate sexes):**
  - Most animals, some plants (willows...)

**Sex determination / differentiation**

**Sex determination:** Initial event that determines whether the gonads will develop as testes or ovaries.

**Sex differentiation:** Subsequent events that ultimately produce either the male or female sexual phenotype.

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**Diagram:**

- **Determination:**
  - Intermediate Mesoderm → Bipotential Gonad → Testis or Ovary
  - SOX9 → DAX-1
  - SRY → Testis

- **Differentiation:**
  - Testis:
    - WT1, SF-1 → Sertoli Cells → MIS → Müllerian Duct Regression
    - MIS-R → Male Internal and External Genitalia
  - Leydig Cells → Testosterone → Male Internal and External Genitalia
  - Ovary: Female Sex Differentiation
  - WT1, SF-1 → Ovary
  - DAX-1 → Ovary

Sex determination (1)

**Mechanism:**
Developmental decisions that occur during embryogenesis

**Aristotle (ca. 335 B.C.):**
Sex is determined by “the heat of the male partner during intercourse”

**Vesalius (~ 1543) held the same view**

**During the 1600s and 1700s:**
Females were seen as producing eggs that could transmit parental traits, and the physiology of sex organs began to be studied

**Until 20th century:**
The environment – temperature and nutrients, in particular – was believed to be important

Factors favoring the storage of energy and nutrients predisposed one to have female offspring, whereas factors favoring the utilization of energy and nutrients influenced one to have male offspring (Geddes and Thomson, 1890)
Sex determination (2)

In 1900:
Rediscovery of Mendel’s work

In 1902:
Rediscovery of the sex chromosomes (McClung)

In 1905:
Establishment of the correlation (in insects) of the female sex with XX sex chromosomes and the male sex with XY or XO chromosomes (Stevens; Wilson)

- A specific nuclear component is responsible for directing the development of the sexual phenotype
- Evidence accumulated that sex determination occurs by nuclear inheritance rather than by environmental influence

Today:
Both environmental and internal mechanisms of sex determination can operate in different species
Sex determination (3)

Set conditions for deciding which way switch will be thrown

MASTER SWITCH

or

ON

Downstream regulatory factors induced

New development pathway induced

OFF

Default regulatory factors operate

Default developmental pathway maintained
A. Environmental sex determination

Temperature-dependent sex determination (reptiles)

Sex of most snakes and most lizards →
sex chromosomes
Sex of most turtles and all species of crocodilians →
environment (temperature)

Location-dependent sex determination

![Graph showing sex ratio (% male) vs. temperature (°C) for different species: Trachemys scripta, Macrochelys temminckii, and Alligator mississippiensis.](image)
B. Physiological/social sex determination

Body size:
Some fish are first male then female
C. Chromosome constitution and sex determination (1)

- Sex determination is associated with sex chromosomes that are different between male and female individuals.
- Many species have heteromorphous sex chromosomes (different in size and gene content).
- Three important chromosomal sex-determining systems:
  - XX – XY system
  - XX – XO system (i.e. non-heteromorphous)
  - ZZ – ZW system

- Drosophila
- Mammals
- Some insects (e.g. bees, wasps, ants)
- Butterflies, most birds, some fish, amphibians

= inherited from male parent
= inherited from female parent
C. Chromosome constitution and sex determination (2)

Sex is determined by the action of genes but is also often a chromosomal trait.

**Sex chromosomes** – (X and Y) – are involved in determining sex and other traits (X-linked traits). They are **heteromorphic**.

**Autosomes** – the non-sex chromosomes
- ♀ = XX, the **homogametic** sex
- ♂ = XY, the **heterogametic** sex

**XX/XY** mode of sex determination

Examples:
- Human (2n = 46) = 22 homologous pairs of autosomes + one pair of sex chromosomes
- *Drosophila* (2n = 8) = 3 homologous pairs of autosomes + one pair of sex chromosomes

Alternative types of chromosomal sex determination mechanisms:
- ♀ = XX, the homogametic sex
- ♂ = XY, the heterogametic sex
- ♀ = X, the heterogametic sex
- ♂ = ZZ, the homogametic sex

**XX/X0** mode of sex determination (some insects)

**ZW/ZW** mode of sex determination (butterflies, most birds, some fish and amphibians)

In **XX/XY**, **XX/X0**, and **ZW/ZW** systems, the sex of the offspring is determined by the chromosome content of the gamete from the heterogametic sex.
C. Chromosome constitution and sex determination (3)

Ploidy and sex determination

Hymenopterans (bees, wasps, ants): Sex is related to the number of sets of chromosomes (females 2N; males N)

Females (queen, workers) are diploid, males (drones) are haploid

Fertilized eggs develop into females
Unfertilized eggs develop into males (males have no fathers)
Dosage compensation (1)

- Number of active genes should be the same

Xist: methylation

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Drosophila</th>
<th>Caenorhabditis elegans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female : Male</td>
<td>Female : Male</td>
<td>Female : Male</td>
</tr>
<tr>
<td>2 : 1</td>
<td>2 : 1</td>
<td>2 : 1</td>
</tr>
</tbody>
</table>

X-chromosome inactivation

Transcripts

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Drosophila</th>
<th>Caenorhabditis elegans</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 1</td>
<td>1 : 1</td>
<td>1 : 1</td>
</tr>
</tbody>
</table>

Male – No Barr Bodies

Female – Barr Bodies

Barr body
The Lyon Hypothesis

2. Which X is inactivated?
- Mary Lyon proposed the mechanism of X inactivation in mammals
- based on studies of female mice for X-linked coat color genes
- X inactivation occurs around the 16th day of embryonic development
- is random in each cell with respect to which X is initially inactivated
- once an X is inactivated however, all descendant cells in that particular cell line inactivate the SAME X

3. X-inactivation leads to genetic mosaics
- females who are heterozygous for X-linked genes will contain two types of cell clones
  - Maternal X is inactivated in 1/2 of cell lines
  - Paternal X is inactivated in 1/2 of cell lines
- heterozygous females can therefore show two different phenotypes in a mosaic pattern
Dosage compensation (3)

Calico Cats

- can only be females
- only females can be heterozygous for coat color gene on the X chromosome
- one allele codes for orange; the other allele codes for black
- 1/2 of cells have inactivated the black allele--results in patches of orange fur
- 1/2 of cells have inactivated the orange allele--results in patches of black fur
- males are either black or orange, because they are hemizygous for the X-linked color gene
Mechanism of X-inactivation

-XIC = X-inactivation center
  located on the q arm of the X chromosome
-Xist gene = X inactive specific transcript
  Located within XIC region
  RNA product from this gene does not leave the nucleus, so it is not translated into protein
  Instead RNA acts as a “cage” that envelopes the X destined to be inactivated
  Further transcription of this RNA on the active X is repressed by methylation of the gene’s promoter region; also true for X in males
Dosage compensation (5)

![Diagram showing dosage compensation](image-url)

**Diagram Explanation:**
- **X-inactivation center (XIC):**
  - Repressor of inactivation binds to one X chromosome.
  - Inactivation spreads out from the XIC of the X chromosome that has not bound the repressor.
  - One X chromosome is inactivated.

**RNA Remains in Nucleus and Binds to the Inactive X Chromosome:**
- Inactive X: 17 Kb RNA, XIST
- Active X: No RNA, HPRT

**RNA to Cytoplasm:**
- No RNA

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Drosophila

- Main difference in genital structure
- Smaller: bristle, pigmentation, sex comb
- Induced by binary gene switches
Sex is determined by X-chromosomes (not by Y, as in humans, but determined by X:A ratio)

- **Sxl** only turned on in **XX**
**Sex-determination in *Drosophila*** (2)

**X:A BALANCE**

- Rules of sex determination in *Drosophila*
  - $X:A \text{ ratio } \geq 1.0 = \text{ Female}$
  - $X:A \text{ ratio } \leq 0.5 = \text{ Male}$
  - $X:A \text{ ratio } > 0.5 \text{ but } < 1.0 = \text{ Intersex}$

**Drosophila X:A Balance**

- Genes that determine “male” are on autosomes.
  - Males result because they lack a second X;
  - Y chromosome is inert (needed for fertility).

- Some genes that determine “female” are on the X chromosome, but it takes the proper X:A ratio to activate these genes.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of X chromosomes</th>
<th>No. of sets autosomes</th>
<th>X:A ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>2</td>
<td>2</td>
<td>2/2=1.0</td>
</tr>
<tr>
<td>(normal female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XY</td>
<td>1</td>
<td>2</td>
<td>1/2=0.5</td>
</tr>
<tr>
<td>(normal male)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XXX</td>
<td>3</td>
<td>2</td>
<td>3/2=1.5</td>
</tr>
<tr>
<td>(metafemale)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XY</td>
<td>1</td>
<td>3</td>
<td>1/3=0.33</td>
</tr>
<tr>
<td>(metamale)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XX</td>
<td>2</td>
<td>3</td>
<td>2/3=0.67</td>
</tr>
<tr>
<td>(intersex)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Female Sex lethal (Sxl)

- **Ratio X:A**  
  \(A = \text{autosome}\)

- **Transcription Sxl**

- **Later: Sxl maintains own expression and acts as splicing factor**

  \(\rightarrow \text{functional Sxl}\)
Male Sex lethal (Sxl)

- **Early:**
  - no Sxl transcription
  - not enough X-linked transcription factors

- **Late:**
  - no Sxl to exclude exon 3 termination codon in exon 3

$Sxl^m$: 48 aa  
(Sxl$^f$: 354 aa)
From splicing to (Double)sex

Sxl regulates sex determination
- induces skipping of exon 2 in Transformer (Tra)
- male or female Tra
# Role of Sxl in *Drosophila*

<table>
<thead>
<tr>
<th>XX</th>
<th>XY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex-lethal establishment promoter (Sx/P_e)</strong></td>
<td><strong>Sex-lethal establishment promoter (P_e)</strong></td>
</tr>
<tr>
<td>ON</td>
<td>OFF</td>
</tr>
<tr>
<td><strong>Sex-lethal maintenance promoter (Sx/P_m)</strong></td>
<td><strong>Sex-lethal maintenance promoter (Sx/P_m)</strong></td>
</tr>
<tr>
<td>ON</td>
<td>ON</td>
</tr>
</tbody>
</table>

- **early Sxl protein**
- **late Sxl protein**
- **productive splicing**
- **trigger to start feedback loop**

**Female** 

- **no early Sxl protein**
- **nonproductive splicing**
- **no late Sxl protein**

**Male**
Role of Doublesex (Dsx)

**Tra present**

Female splicing of Dsx
- Activation of female-specific genes (yolk proteins)
- Inhibition of male-specific genes

**Tra not present**

Male splicing of Dsx
- Inhibition of female-specific genes
- Activation of male-specific genes

Dosage Compensation in *Drosophila*

Hyperactivation of X chromosomes
- Dosage compensation does not involve inactivation of an X in females
- Single male X chromosome is hyperactivated
  - SxI gene off → repressor proteins
  - Proteins form a complex that binds to single X in males
  - Hyperactivates or doubles the amount of transcriptional activity of X-linked genes on the male's single X chromosome
Sex determination in mammals

Removed indifferent gonads from rabbits *in utero*

Sexual differentiation path of all: female

**Conclusion:** testes produce substances required for male embryos to develop
Testosterone (T) induces the Wolffian ducts to differentiate into epididymides, vasa deferens, and seminal vesicles.

Anti-Müllerian hormone (AMH; Müllerian-inhibiting substance, MIS) causes regression of the Müllerian ducts, which in its absence would normally develop into the Fallopian tubes, uterus and upper vagina as is observed in female embryos.

First rule of sex determination: The development of the gonads into testis or ovary determines the sexual differentiation of the embryo.

Second rule of sex determination: The Y chromosome carries the sex-determining information in mammals.
Sex determination in mammals (3)

Three levels of sexual dimorphism

1. Genetic sex
   - XY
   - XX

2. Gonadal sex
   - Testes
   - Ovaries

3. Phenotypic sex
   - Male
   - Female

Genetic sex ➔ Gonadal sex ➔ Phenotypic sex
Sex determination in mammals (4)

XY SYSTEM IN MAMMALS

sex is determined by presence of the Y chromosome

"female" is the default sex; due to absence of the Y chromosome

<table>
<thead>
<tr>
<th>Sex</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>XX</td>
<td>Normal female</td>
</tr>
<tr>
<td>Male</td>
<td>XY</td>
<td>Normal male</td>
</tr>
<tr>
<td>Female</td>
<td>XO</td>
<td>Turner syndrome (sterile)</td>
</tr>
<tr>
<td>Male</td>
<td>XXY</td>
<td>Klinefelter syndrome (sterile)</td>
</tr>
<tr>
<td>Female</td>
<td>XXX</td>
<td>Superfemales (may be sterile)</td>
</tr>
<tr>
<td>Male</td>
<td>XYY</td>
<td>Supermales (may be sterile)</td>
</tr>
</tbody>
</table>

Why Y?

Action of a sex-determining gene on the Y chromosome

TDF -- testis determining factor

- product of SRY gene

sex-determining region on the Y chromosome

TDF stimulates embryonic gonads to begin producing testosterone

Testosterone stimulates differentiation of embryonic gonadal tissue into “male” structures

Female tissues develop when SRY gene is absent

- **XXY: Klinefelter syndrome**
  - same phenotype when testosteron receptor is defect

- **Turner syndrome: one X-chromosome**
  - women produce no eggs

17 year-old male with Klinefelter
Testis-determining factor (1)

Transplantation studies using isogenetic mice (~ homozygous for each locus)

Only difference: XY or XX

Transplanted tissue from male into female →
Immune response (H-Y antigen)

Also found in other mammalian species

No confirmation in individuals with sex reversal
Testis-determining factor (2)

**SRY** (sex determining region on the Y chromosome)

**ZFY** (zinc finger on Y chromosome)

H-Y antigen
SRY is the Male Determination Factor

HMG Transcription Factor
Binds to sry element of Sertoli cell MIS/MRF gene (stimulates) & of Leydig cell P450 aromatase gene (inhibits) [1993]

Cytogenetic localization of SRY region (after McLaren)

Sry (14 kb DNA fragment)

Transfection of Sry sequence in the mouse successfully transforms an XX embryo to a male, demonstrating the Sry gene is necessary and sufficient to determine maleness in a mammal (Koopman et al., Nature 351:117-121, 1991.)

Birds and reptiles
No SRY

Platypus
No SRY?

Kangaroo
SRY

Human
SRY

Mouse
Sry

Mole
vole
No Sry

SRY
Modified
30 Myr
80 Myr

130 Myr
SRY evolved
170 Myr

Y chromosome evolved

310 Myr

TRENDS in Genetics
SRY (4)

Diagram showing the role of SRY in sex determination:

- Male XY: Gonads develop into testes under the influence of testosterone, leading to male reproductive tract and internal genitalia.
- Female XX: Gonads develop into ovaries, and the Müllerian ducts regress, leading to female genitalia.
- Müllerian-inhibiting factor:
  - Male: Müllerian ducts regress under the influence of DHT.
  - Female: Müllerian ducts regress under the influence of testosterone and lack of Müllerian-inhibiting factor.
- **SRY** (5)

**Y chromosome contains genetic determinant (SRY) of gonadal differentiation**

- **SRY** is expressed in pre-Sertoli cells
- Fetal testis produce testosterone (T) and AMH
- Fetal T secretion initially under control of placental hCG
- **T** converted to DHT by 5α-reductase
In the absence of SRY, undifferentiated gonads develop into ovaries.

In absence of T → Wolffian ducts regress and external genitalia develop into female phenotype.

In the absence of AMH → Müllerian ducts develop into female reproductive tract.
Reproductive dysfunction

XXY (Klinefelter’s syndrome)
Male phenotype

XO (Turner’s syndrome)
Female genotype

5α-reductase deficiency
Normal male internal genitalia but incomplete masculinization of external genitalia at birth

Androgen insensitivity (AI)
Complete AI can lead to female phenotype with no internal genitalia (Wolffian ducts do not develop and Mullerian ducts regress)

Congenital adrenal hyperplasia (CAH)
Elevated fetal androgen in females with severe CAH causes development of both Wolffian and Mullerian ducts
Gene interactions in gonadal development
KL Parker, A Schedl, BP Schimmer

Sex with two SOX on: SRY and SOX9 in testis development
MJ Clarkson, VR Harley
Summary: determination of sexual phenotype

**Mammals**

- **Female (XX)**
  - One X inactivated via Xist
  - gonad
  - Wolffian duct degenerates;
  - Mullerian duct forms oviduct
  - female secondary sexual characteristics

- **Male (XY)**
  - Sry active on Y chromosome
  - gonad
  - testis
  - Wolffian duct forms vas deferens;
  - Mullerian duct degenerates
  - male secondary sexual characteristics

- female external genitalia
- male external genitalia
- undifferentiated genital region

- no testosterone; female gonadal hormones
- testosterone
<table>
<thead>
<tr>
<th>Drosophila</th>
<th>C. elegans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong>&lt;br&gt;XX</td>
<td><strong>Male</strong>&lt;br&gt;YX</td>
</tr>
<tr>
<td>Sxl on</td>
<td>Sxl off</td>
</tr>
<tr>
<td>sex-specific splicing of double-sex RNA</td>
<td>fruitless</td>
</tr>
<tr>
<td>development of female reproductive organs and somatic sexual phenotype</td>
<td>development of male reproductive organs and somatic sexual phenotype</td>
</tr>
</tbody>
</table>

**Hermaphrodite**<br>XX

**Male**<br>YX

<table>
<thead>
<tr>
<th>Increased transcription</th>
<th>Reduced transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>development of Hermaphrodite reproductive organs and somatic sexual phenotype</td>
<td>development of male reproductive organs and somatic sexual phenotype</td>
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</table>

**Male**<br>YX

<table>
<thead>
<tr>
<th>XO lethal off</th>
<th>XO lethal on</th>
</tr>
</thead>
<tbody>
<tr>
<td>sexual behavior</td>
<td>transformer-1 high</td>
</tr>
<tr>
<td>transformer-1 low</td>
<td>development of male reproductive organs and somatic sexual phenotype</td>
</tr>
</tbody>
</table>
Primordial germ cell (PGC) migration

Often germ cells develop away from gonads

- Protection against signals for body plan formation?
- Selection for the ‘best’?