Lab. 11

Barr body:

X Chromosome Inactivation
<table>
<thead>
<tr>
<th>Organism</th>
<th>No. chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>46</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>48</td>
</tr>
<tr>
<td>Dog</td>
<td>78</td>
</tr>
<tr>
<td>Horse</td>
<td>64</td>
</tr>
<tr>
<td>Chicken</td>
<td>78</td>
</tr>
<tr>
<td>Goldfish</td>
<td>94</td>
</tr>
<tr>
<td>Fruit fly</td>
<td>8</td>
</tr>
<tr>
<td>Mosquito</td>
<td>6</td>
</tr>
<tr>
<td>Nematode</td>
<td>11(m), 12(f)</td>
</tr>
<tr>
<td>Horsetail</td>
<td>216</td>
</tr>
<tr>
<td>Sequoia</td>
<td>22</td>
</tr>
<tr>
<td>Round worm</td>
<td>2</td>
</tr>
</tbody>
</table>
What Exactly is a chromosome?

- Chromosomes are the rod-shaped, filamentous bodies present in the nucleus, which become visible during cell division.
- They are the carriers of the gene or unit of heredity.
- Their number can be counted easily only during mitotic metaphase.
- The term “Chromosome”, however, was first used by Waldeyer in 1888.
- They were given the name chromosome (Chromo = colour; Soma = body) due to their marked affinity for basic dyes.
- This X-Y system of mammals is not the only chromosomal mechanism of determining sex.

- Other options include the X-0 system, the Z-W system, and the haplo-diploid system.
• 1940’s two Canadian scientists noticed a dark staining mass in the nuclei of cat brain cells.
• Found these dark staining spots in female but not males.
• This held for cats and humans
• They thought the spot was a tightly condensed X chromosome.
During embryonic development one X chromosome becomes inactive (Barr Body).

All mitotic divisions create cells with the same inactive X.

The inactive X chromosome are normally found only in female somatic cells.
Sex Chromosomes:

- Females XX, males XY
- Females have two copies of every X-linked gene; males have only one.

How is this difference in gene dosage compensated for? OR

- How to create equal amount of X chromosome gene products in males and females?
• **(gene expression)** Levels of enzymes or proteins encoded by genes on the X chromosome are **the same in both males and females**

• Even though males have **1 X chromosome** and females have **2**.
Dosage Compensation

• To compensate for females having 2 X chromosomes vs. males having only 1 X. chromosome could do one of two things:

  Double the amount of transcription of X-chromosome genes in males.

  OR

  Inactivate one of the X-chromosomes in females.→→→ Barr Bodies
Barr bodies represent the inactive X chromosome and are normally found only in female somatic cells.
No. Barr bodies = N - 1  
(N = # X chromosomes present)

<table>
<thead>
<tr>
<th>Chromosome Configuration</th>
<th>Number of Barr Bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>46, XX</td>
<td>1 Barr body</td>
</tr>
<tr>
<td>45, X</td>
<td>0 Barr body</td>
</tr>
<tr>
<td>47, XXY</td>
<td>1 Barr body</td>
</tr>
<tr>
<td>47, XXX</td>
<td>2 Barr bodies</td>
</tr>
<tr>
<td>48, XXXX</td>
<td>3 Barr bodies</td>
</tr>
</tbody>
</table>
A woman with the chromosome constitution 47, XXX should have 2 Barr bodies in each cell.

XXY individuals are male, but have a Barr body.

XO individuals are female but have no Barr bodies.
Example:

- **G6PD**, glucose 6 phosphate dehydrogenase, gene is carried on the **X chromosome**.
- This gene codes for an enzyme that breaks down sugar which helps red blood cells work properly.
- 🔄 **normal Females** produce the same amount of G6PD enzyme as **normal males**.
- 🔄 **XXY and XXX** individuals produce the same amount of G6PD as anyone else.
• In cells with more than two X chromosomes, only one X remains genetically active and all the others become inactivated.

• In XXX and XXXX females and XXY males only 1 X is activated in any given cell the rest are inactivated.

• In some cells the paternal allele is expressed.

• In other cells the maternal allele is expressed.
X inactivation

Fertilized ovum

Mitosis

Random inactivation of one X chromosome

Mitosis

Maternal X inactivated (Paternal X expressed)

Maternal X inactivated (Maternal X expressed)

Paternal X inactivated (Maternal X expressed)

Paternal X inactivated (Paternal X expressed)
• Which chromosome is inactive is a matter of chance (random inactivation), but once an X has become inactivated, all cells arising from that cell will keep the same inactive X chromosome.

• In human embryos, sex chromatin bodies have been observed by the 16th day of gestation.
Mechanism of X-chromosome Inactivation

• A region of X chromosome near the centromere called the **X-inactivation center (XIC)** is the control unit.

• This region contains the gene for **X-inactive specific transcript (XIST RNA)**.

• The Xist gene is the **only gene** which is **expressed** from the X **inactivated** but not from the X **activated**.
• The **silencing of genes** along the Xi occurs soon after coating by Xist RNA. *(repressive)*

• The **Xi** has high levels DNA methylation locks the chromosome in the inactive state. *(associated with gene silencing)*
stabilizes the cell membrane by binding divalent cations (Mg++, Ca++) and inhibits nuclease activity that destroys the cell's RNA. This gives osmotic shock that leads to the rupture of cell wall and membrane.

In this laboratory exercise, the E. coli bacterial cells are resuspended in buffer containing:

- **EDTA**
- **RNase**

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**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.
Methylation has two of the requirements for mechanism of determination:

1. It represses gene activity
2. It is permanent.
X-inactivation facts about calico cats

- 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
A diagram illustrates the genetic inheritance of coat color in cats. The diagram shows a non-orange male and an orange female, with possible offspring combinations labeled as calico females and orange males. The genetic symbols represent:

- **Y**: no color genes
- **X**: chromosome with orange gene
- **XX**: chromosome with non-orange gene

The diagram delineates the possible genetic combinations resulting in different coat color expressions, such as orange and calico.
What determines maleness and femaleness in humans?

2 possibilities:

1. Two X chromosomes are needed to make a female. If you have one X chromosome then you default to male. **No, not the mechanism.**

2. The Y chromosome is needed to make a male. Lack of the Y chromosome and you default to female. **Yes, this is the mechanism.**
- Presence of Barr bodies in a buccal smear was introduced by the International Olympic Committee Medical Commission as a sex determination (gender verification) test in 1968.

- This test, known as the Barr test or buccal smear sex test, was responsible for excluding about one female competitor in 400 from international competition.

- At the 1992 Barcelona Olympics, the Barr test was replaced by the polymerase chain reaction test.
Because,

• Someone with a mutation in the SRY gene can develop into a female even though there are SRY and the Y chromosome are present.

• In rare cases, the SRY gene can be transferred to the X chromosome by chromosomal crossover during the production of sperm, and the resulting XX individual would be phenotypically male.
Testicular feminization syndrome (TFS),
A genetic condition in which an XY (male) zygote develops as a phenotypically female adult, due to failure of androgen receptors (Androgen insensitivity syndrome AIS).
Dora Ratjen (November 20, 1918 Bremen, Germany - April 22, 2008) was a German athlete who competed for Germany in the Women's High Jump at the 1936 Summer Olympics at Berlin, finishing fourth, but was later discovered to be male.
Stella Walsh won a silver medal at the 1936 Olympics - but was later found to be a man.

Stella Walsh was killed in a random act of violence in 1968. As part of a routine autopsy, coroners discovered that Stella was a masculine pseudohermaphrodite (mosaicism).
Semenya (south Africa) told to take gender test

800 meter gold medal in Berlin 2009

Reports in two newspapers in September said the results of the tests showed Semenya has both male and female characteristics. The IAAF has declined to confirm those reports.
Blood Smear

Collection and Preparation

1. Sterilize finger using 70% alcohol
2. Puncture the area with a sterile blood lancet (firm and quick stab)
3. Wipe of the first drop of blood
4. Prepare the blood smear on a clean slide using the two-slide method
5. Air dry
Fixing and Staining of Blood Smears

1. Fix the prepared blood smear by immersing the slide in Methanol for 5 seconds.
2. Air dry
3. Stain the blood smear
   a. Dip slide in eosin (solution 2) for 3 seconds
   b. Transfer the slide in Methylene Blue (solution 3) for 6 seconds or until the color of the smear turns blue
   c. Wash using the prepared washing buffer
   d. Blot dry
4. Add a small drop of oil immersion solution on the blood smear and examine under OLO. Look for Barr bodies among polymorphonuclear neutrophils
5. Draw and identify Barr bodies
A. Human, air-dried smear, Wright's stain, 1416 x.
B. Human, buccal epithelium scraping, aceto-orcein stain, 500 x.
C. Human corpus luteum cells, 10% formalin, H. & E., 1416 x.
اذكروا ما هو أفضل ذكرًا قياسًا
ذلك كثيرًا

إذا أردت أن يطمئن قلبك، فما عليك إلا بذكر

DAWAH.WS