Genetics & molecular biology

◉Sheet ○Slide

Number:	-17
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Gregor Mendel's Laws:

1. **Law of Segregation**: each homologous chromosome will separate

and appear in different daughter cells, such that each gamete

receives one allele for a given trait

2. **Law of Independent Assortment**: the arrangement of the paired chromosomes with respect to the poles of the spindle apparatus is random along the metaphase plate. As the paternal and maternal chromosomes in a homologous chromosome is not identical, the number of possible arrangements is 2^n where n represents the number of chromosomes. Humans have 23 different chromosomes, so the number of possible combinations is over 8 million.

In conclusion: Meiosis generates genetic diversity through

- The exchange of genetic material between homologous chromosomes during meiosis 1
- The random alignment of maternal and paternal chromosomes in meiosis 1
- The random alignment of the sister chromatids at meiosis 2

Now the question is, WHY WE STUDY CHROMOSOMES?

Simply the knowledge of chromosomes is important clinically. Many clinical conditions are linked to chromosomal abnormality and approximately 0.6-1% of all newborns in humans have a chromosomal abnormality.

These chromosomal abnormality accounts for about:

1 – 20-27% of individuals having sex reversal or pubertal anomalies

2 – up to 70% of spontaneous miscarriages

3 – 2-3% of couples having a history of multiple miscarriages.

Note: numbers are not for memorizing.

How to study chromosomes

First a tissue must be extracted to be studied. The type of tissue depends on the **type the disease** and the **purpose** of the study.

For example:

1 - Peripheral blood (lymphocytes) is the most common tissue used in chromosomal studies. Remember that RBCs don't have nucleus so there is no DNA in it. It is important to know that cells that are examined must be capable of proliferation. The most **accessible** cells that meet this requirement are WBC and therefore they are mostly used.

2 – Bone Marrow is used to study chromosomes for those having disease in blood

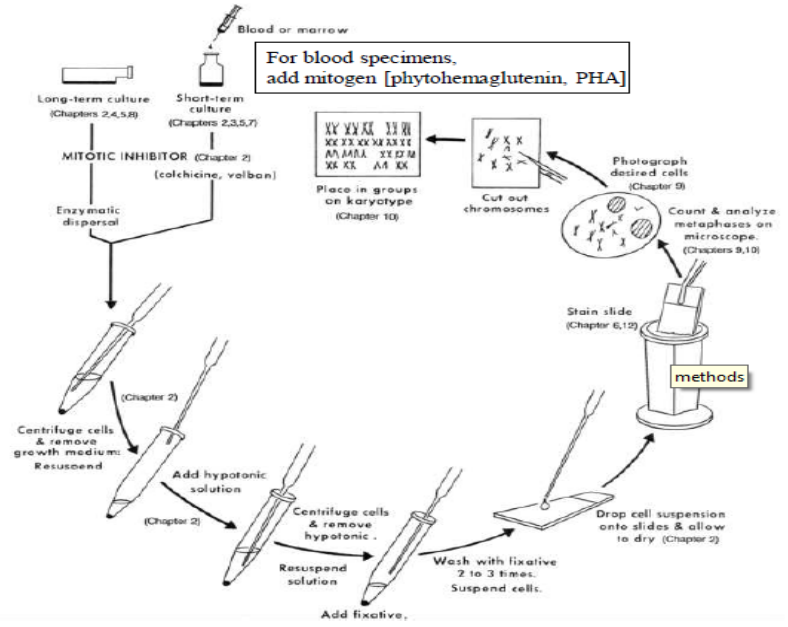
3 – Amniotic fluid cells is used to study embryonic genes

Other sources: chorionic villi and skin or organ biopsy

After determining which tissue we must use, know it is time to show the chromosomes and study them:
The concept of the process is easy. Remember that chromosomes are visible in the **M Phase** of the cell cycle and the majority of cells that are obtained from the tissue are either at rest (G0) or in the interphase. So, cells must be **induced** to enter the cell cycle and reach the M phase then **stopped** there for studying.

Primary Steps for Culture Establishment and Harvest of Specimens

- **Add Mitogen (when needed)**
- **Hypotonic Swelling**
- **Fixation**
- **Analysis**



Steps to produce a karyotype:

1 – blood is taken and placed in a petri dish or flask with media (sterile water with nutrients). The media also contains a **mitogen** (it is a chemical that induces the cell to enter the cell cycle) usually *phytohemaglutenin*.

2 – another chemical compound is added, usually *colchicine*, to stop the cells at the M phase, specifically at the metaphase.

3 – the cells are centrifuged to remove the media.

4 – a hypotonic solution is added. The solution enters the cells and make them swollen and fragile.

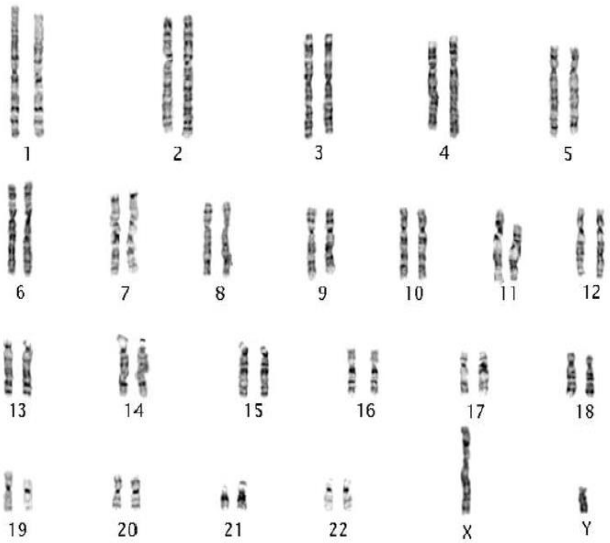
5 – suspended cells are dropped onto slides. Cells burst open, and chromosomes will be scattered on the slide.

6 – Giemsa stain is added to show the chromosomes.

A karyogram (karyotype)

After staining with Giemsa stain, each chromosome will have different length and a special staining that produce different banding patter. Chromosomes are then arranged according to their length to produce a karyotype

Karyotype: is a photograph or a diagram of an ordered arrangement of chromosomes from cells that are placed in a standard order (generally by length, chromosome 1 is the longest and 22 is the shortest followed by the sex chromosomes)



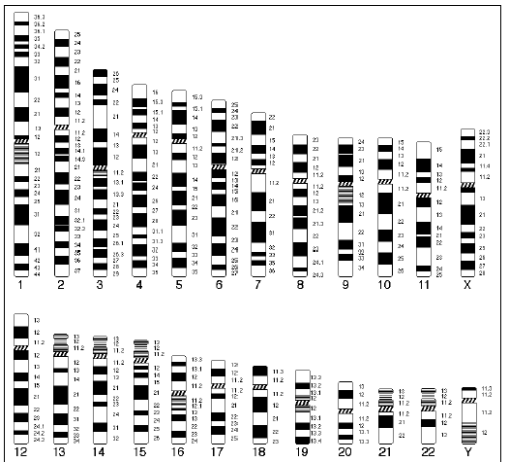
(The picture is taken in the metaphase because that's when the chromosomes are most condensed)

Note: each band\chromosome is actually 2 sister chromatids !! ask yourself why!

What is **ideogram**?
ideogram is diagrammatic representation of the karyotype

Notice in the picture there are 24 different chromosomes (22 autosomal and the 2 sex chromosomes) the ideogram is a representation of the different banding patterns of the chromosomes which we use to distinguish the chromosomes to make the karyotype

note: each chromosome has different banding pattern (dark and light bands)



the location of the dark band or the light band for the same chromosome is different among different types of banding methods. There are (G,R,C,Q,T banding) we're to required t know G and R banding only

G-banding

Dark bands (heterochromatin) tends to be condensed, AT-rich DNA and gene-poor region. Remember that A is attached to T be 2 hydrogen bonds only so there is enough space for Giemsa stain

Light bands (euchromatin) tends to be de-condensed, GC rich and gene-rich regions. G and C are connected by 3 H bonds so there is no enough space for Giemsa stain.

This method normally produces 300-400 bands among the 23 pairs of human chromosomes. Each band represents several million to 10 million base pairs of DNAs.

R-banding

It is the reverse of G-banding. The dark regions are euchromatin (GC rich) and the light regions are heterochromatin (AT rich)

This banding pattern is produced by heating the chromosomes before applying Giemsa stain. Heat will destroy the AT-rich region (only 2 bonds) and weaken the GC-rich region (3 bonds) so only GC-rich will take the stain.

When to use it: used with G-banding to determine whether there are deletions of some regions or not.

Also, in some cases R-banding helps confirm the findings of G-banding when there is some bands that aren't clear 100% so flipping the colors might help a bit.

High resolution banding

G and R banding produce about 300-400 bands among the 23 pairs of human chromosomes. Each band represents several million to 10 million base pairs of DNAs. Normally, damage to about 3 million base pairs or more can be detected in the band but damage to a lesser number of base pairs can not be detected. Here comes the high-resolution banding, this method involves the staining of chromosomes during the prophase or prometaphase, *before they reach maximal condensation*. Because chromosomes in these two phases are more extended and less condensed, the number of bands for all chromosomes increases from 300-400 to as many 800 bands.

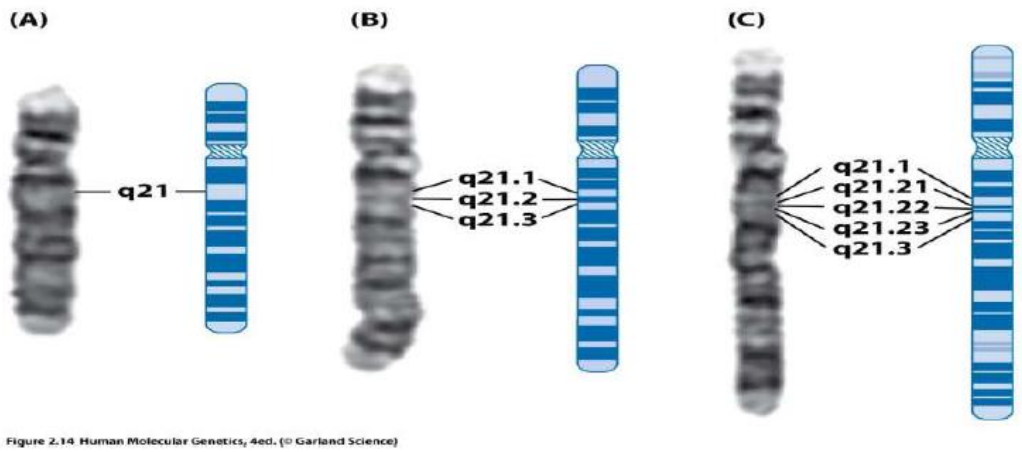


Figure 2.14 Human Molecular Genetics, 4ed. (© Garland Science)

Chromosome shape:

1 – metacentric: the centromere is located in the middle of chromosome

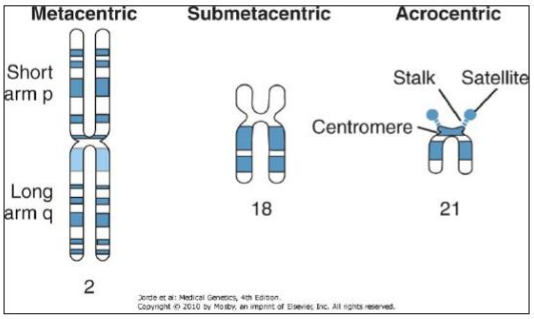
2 – Submetacentric: centromere is displaced from the center

3 – Acrocentric: centromere is placed near the end

note: each chromosome has a short (p) arm and long (q) arm.

Also, each arm is divided into regions having numbers 1,2…

Numbering starts from the centromere (center) toward the telomere (end).

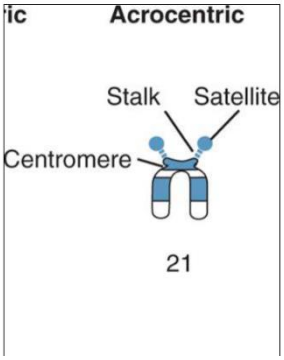


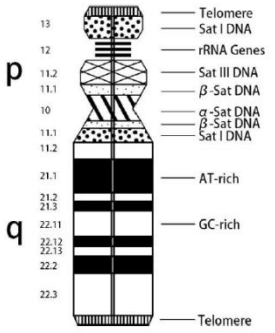
More details about **Acrocentric chromosomes**: (very important)

Acrocentric chromosomes are 13,14,15,21,22. All of them share the following feature (the p arm is the same in all of them)

the P arm contain two distinct regions,

1 – a heterochromatin region that is darkly stained and doesn't codes for gene.it is called **satellite** (only a repetitive sequence of BP)



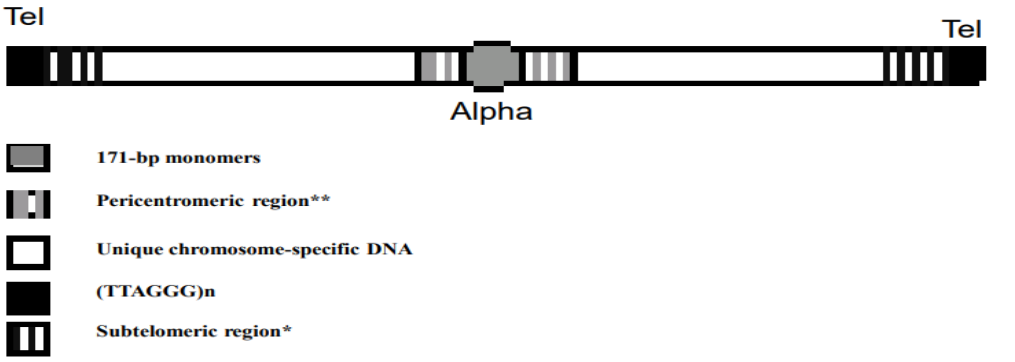


2 – a euchromatin region that is lightly stained and codes for **rRNA**. It is called **stalk**

Note: The P arm of **Acrocentric chromosome** has no clinical significance. So, if there is a mutation\deletion in the p arm of one the **Acrocentric chromosomes,** others can compensate in the production of rRNA.

Structures of chromosomes

Centromere
Telomere
Sub-telomere



1 – Centromere: it a heterochromatin region that contain a tandem repeat of 171 base pairs and proteins (this region is called alpha satellite and all chromosomes have the same alpha satellite in humans) on which spindle fibers attach to. It is required for chromosome **segregation** (if a chromosome doesn't have a centromere it will disappear during cell division and it will not appear in daughter cells), it doesn't have genes but rather it's structural DNA that plays a role during cell division (spindle fibers attach to it)

Around the centromere there is a region called (Pericentromeric region) and then the Unique chromosome-specific DNA appears.

2 – Telomere:

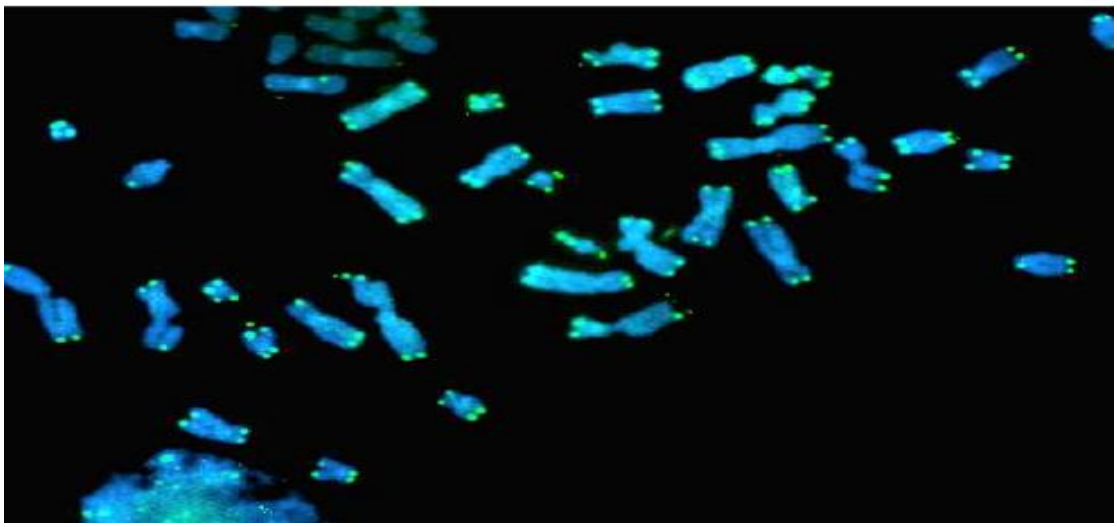
It is a specialized structure at the ends of eukaryotic chromosomes. This region represents a TTAGGG sequence tandemly repeated thousands of times.

It maintains the chromosomal integrity by preventing end-to-end fusion of chromosomes.

A key feature of that region that is not replicated by DNA polymerase and only replicated by an enzyme called **telomerase**. This region is associated with aging as the activity of telomerase decreases with age,

each time a cell divides, the telomeres get shorter. When they get too short, the cell can no longer divide; it becomes inactive or "senescent" or it dies (what is the evolutionary advantage to this? Protection against cancer).

It is also associated with cancer. Cells that are trying to transform into a cancerous cell undergoes a massive round of replications and divisions. As it does so, telomeres are getting shorter and shorter until the cell dies. But if the cell was able to transform into a cancerous cell the activity of telomerase back and the cell now can go an unlimited round of divisions.



3 – sub-telomeric region

This region is common but not identical among all chromosomes.