BIO2133 - GENETICS: MODULE 2 (CHAPTERS 2,7,8,12)

Chromosome Aberrations:

Conclusion

Chromosome aberrations are structural changes in chromosomes that include duplications, deletions, inversions, and translocations. Large segments of the chromosome can be modified by deletions or duplications. Deletions occur when a piece of chromosome is lost, and they can produce serious conditions such as the Cri-du-chat syndrome in humans. Duplications occur as a result of unequal crossing over during meiotic prophase and have been particularly important as a source of redundant or new genes. Several gene families, whose loci have similar structure and function, have confirmed the evolutionary importance of duplications. Inversions and translocations change the gene order along chromosomes and may initially cause little or no loss of genetic information or deleterious effects, aside from alterations of phenotype due to position effects. However, these rearrangements may lead to the production of genetically abnormal gametes during meiosis, resulting in lethality. A "Robertsonian" translocation results in the fusion of the long arms of two chromosomes and loss of the short arms.

You should now be able to:

- List the major categories of chromosomal rearrangement and describe how they can be detected during meiosis.
- Explain the mechanism by which duplications can occur.
- Explain the evolutionary advantages of duplications and inversions.
- Give examples of diseases resulting from chromosomal rearrangements.
- Explain the difference between a paracentric and a pericentric inversion.
- Describe how chromosomal rearrangements can lead to the production of genetically abnormal gametes during meiosis

MODULE TWO LEARNING OBJECTIVES: Should be able to...

a. Describe the molecular anatomy of genes and genomes...

- Explain the meaning of ploidy (haploid, diploid, etc) and how it relates to the number of homologues of each chromosomes.
- Describe how the positions of individual genes on a given chromosome are related to their positions on the homolog of that chromosome.
- Differentiate between a gene and an allele.
- Diagram a typical eukaryotic gene and indicate the locations of (i) regions that are genic but not coding, (ii) regions that are transcribed but not translated, and (iii) regions that are both transcribed and translated.
- Describe the general organization, possible function, and frequency of genes and non-gene DNA sequences in a typical eukaryotic genome.
- Explain the functional significance of packaging DNA into chromosomes and the lack of correlation between chromosome number and genetic information content.
- Explain how all cells have the same genetic content and yet have different functions in the body.

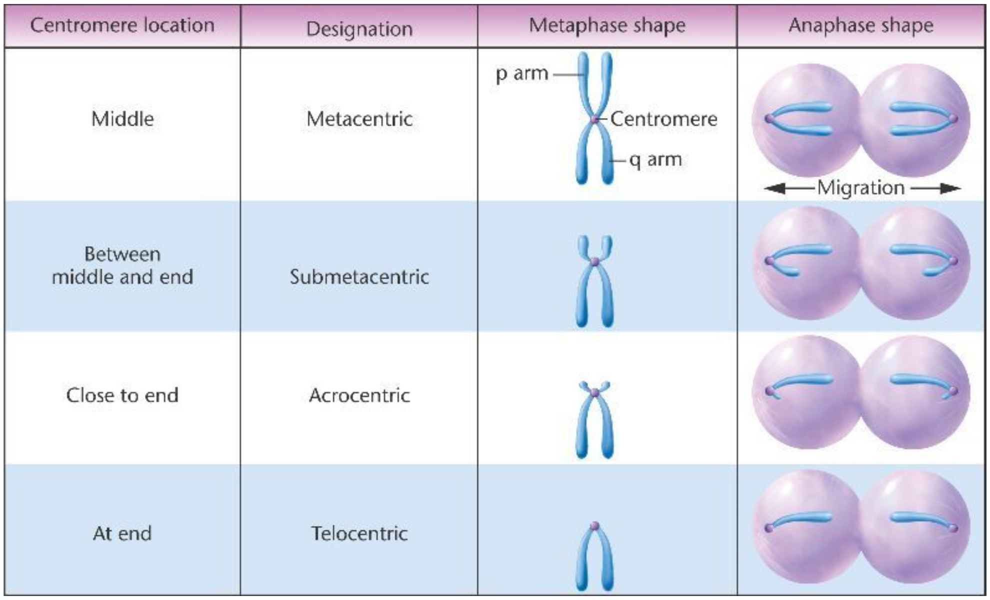
b. Describe the mechasnisms by which an organism's genome is passed on to the next generation...

- Define somatic and germline cells, and list similarities and differences between them.
- Explain why germline mutations can be passed onto the next generation, whereas somatic mutations cannot.
- Describe, using diagrams, the sequence of events involving DNA in meiosis from chromosome duplication through chromosome segregation.
- Distinguish between sister chromatids and homologous chromosomes.
- Explain how independent assortment of alleles during meiosis can lead to new combinations of alleles of unlinked genes.
- Discuss how errors in chromosome number can arise during meiosis, and why such alterations can be detrimental.
- Discuss how sex is determined in humans and other organisms.
- Explain how abnormalities in gene dosage can affect phenotype.
- Explain how organisms compensate for differences in sex chromosome gene dosage between males and females.

Chapter Two - MITOSIS AND MEIOSIS

2.2| Chromosomes Exist in Homologous Pairs in Diploid Organisms

- Chromosomes are most easily visualized during mitosis
- Each chromosome contains a constricted region called the centromere, whose location establishes the general appearance of each chromosome.



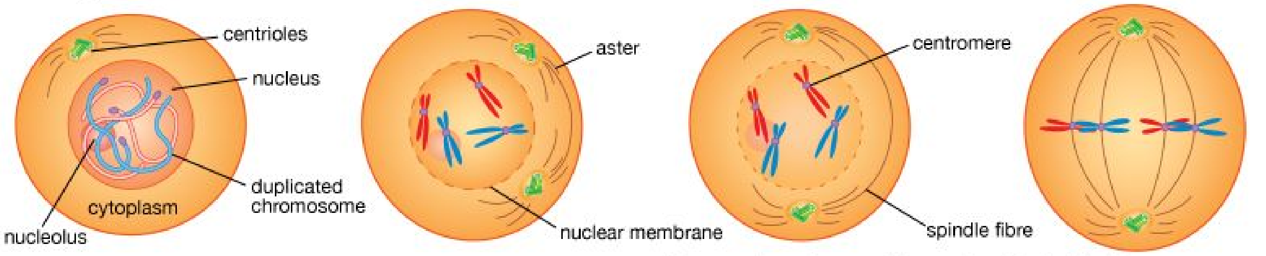
Centromere location	Designation	Metaphase shape	Anaphase shape
Middle	Metacentric	p arm Centromere q arm	Migration
Between middle and end	Submetacentric		
Close to end	Acrocentric		
At end	Telocentric		

- **Chromosomes are classified as metacentric, submetacentric, acrocentric, or telocentric on the basis of the centromere location.**
    - The shorter arm is called the **P arm** (p for petite).
    - The longer arm is shown below the centromere and is called the **Q arm** (next letter in alphabet lol)
    - All somatic cells derived from members of the same species contain an identical number of chromosomes.
        - **Diploid number** in most cases **(2n);** with the exception of sex chromosomes, they exist in pairs with regard to these two properties, and the members of each pair are called: Homologous chromosomes.
            - For each chromosome exhibiting a specific length and centromere placement, another exists with identical features.
        - **Homologous chromosomes;** the pair of chromosomes (not identical) that are next to each other.
        - Many organisms spend their lives in the haploid stage though; they contain only one member of each homologous pair of chromosomes during most of their lives.

        - **Humans have a 2n number of 46 chromosomes, which on close examination exhibit a diversity of sizes and centromere placements.**
        - Each of the 46 chromosomes in the karyotype is clearly a double structure consisting of two parallel sister chromosomes been allowed to continue dividing.
        - **Sister chromatids** are replicas of one another and would have separated into the two new cells as division continued.
    - **A haploid number (n) of chromosomes is equal to one-half of the diploid number.**
        - The genetic information contained in a haploid set of chromosomes constitutes the genome of the species.
        - This includes copies of all genes as well as a large amount of noncoding DNA.
    - **Homologous chromosomes have important genetic similarities**
        - They contain identical gene sites along their lengths; each site is called a **locus.**
        - They are identical in the traits that they influence and in their genetic potential.
        - In **sexually reproducing organisms**, one member of each pair is derived from the **maternal parent (ovum)** and the other is derived from the **paternal parent (sperm)**
        - ***Each diploid organism contains two copies of each gen***e as a consequence of **biparental inheritance;** inheritance from two parents.
        - The members of each pair of genes, while influencing the same characteristic or trait, need not be identical.
        - In a population of members of the same species, many different alternative forms of the same gene; **Alleles,** can exist.
    - During the formation of gametes or spores, ***meiosis converts the diploid number of chromosomes to the haploid number.***
    - **Meiosis = diploid (II:2n) → haploid (I:n)**
        - As a result, haploid gametes or spores contain precisely one member of each homologous pair of chromosome - one complete haploid set.
    - Following the fusion of two gametes at fertilization, the diploid number is reestablished;
        - The **zygote** contains two complete haploid sets of chromosomes.
    - **EXCEPTION:** In many species, one pair, consisting of the sex-determining chromosomes, is often not homologous in size, centromere placement, arm ratio, or genetic content.
        - Exp. in humans, women contain XX chromosomes, mne; XY. These X and Y chromosomes are not strictly homologous. The Y is considerably smaller and lacks most of the gene loci contained on the X.

        - Nevertheless, they contain homologous regions and behave as homologs in meiosis so that gametes produced by males receive either one X or one Y chromosome.
- **Mitosis recap:**
- **Karyokinesis:** ***two daughter cells are formed***, each containing *the same amount of DNA from the parent cell.* Chromosome replicated first, then accurately partitioned.
- **Cytokinesis:** ***cytoplasmic division*** - partitions volume into two parts, then encloses each new cell in a distinct plasma membrane. Organelles replicate themselves as the cytoplasm is reconstituted.

Mitosis, or somatic cell division

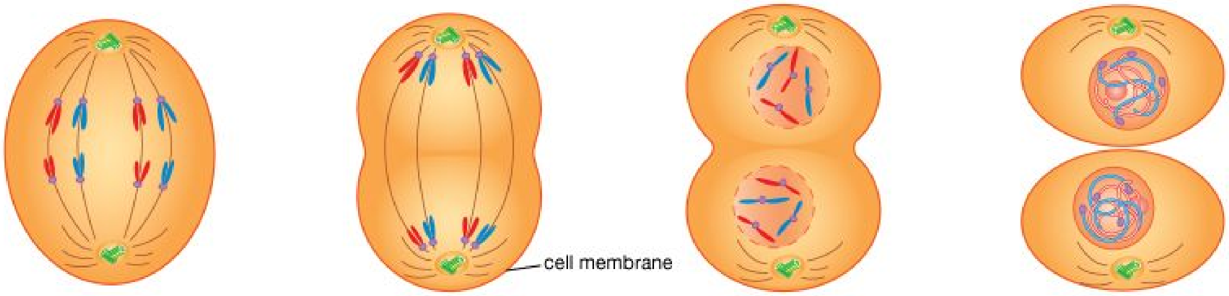


Prior to mitosis, each chromosome makes an exact duplicate of itself. The chromosomes then thicken and coil.

In early prophase the centrioles, which have divided, form asters and move apart. The nuclear membrane begins to disintegrate.

In late prophase the centrioles and asters are at opposite poles. The nucleolus and nuclear membrane have almost completely disappeared.

The doubled chromosomes—their centromeres attached to the spindle fibres—line up at mid-cell in metaphase.

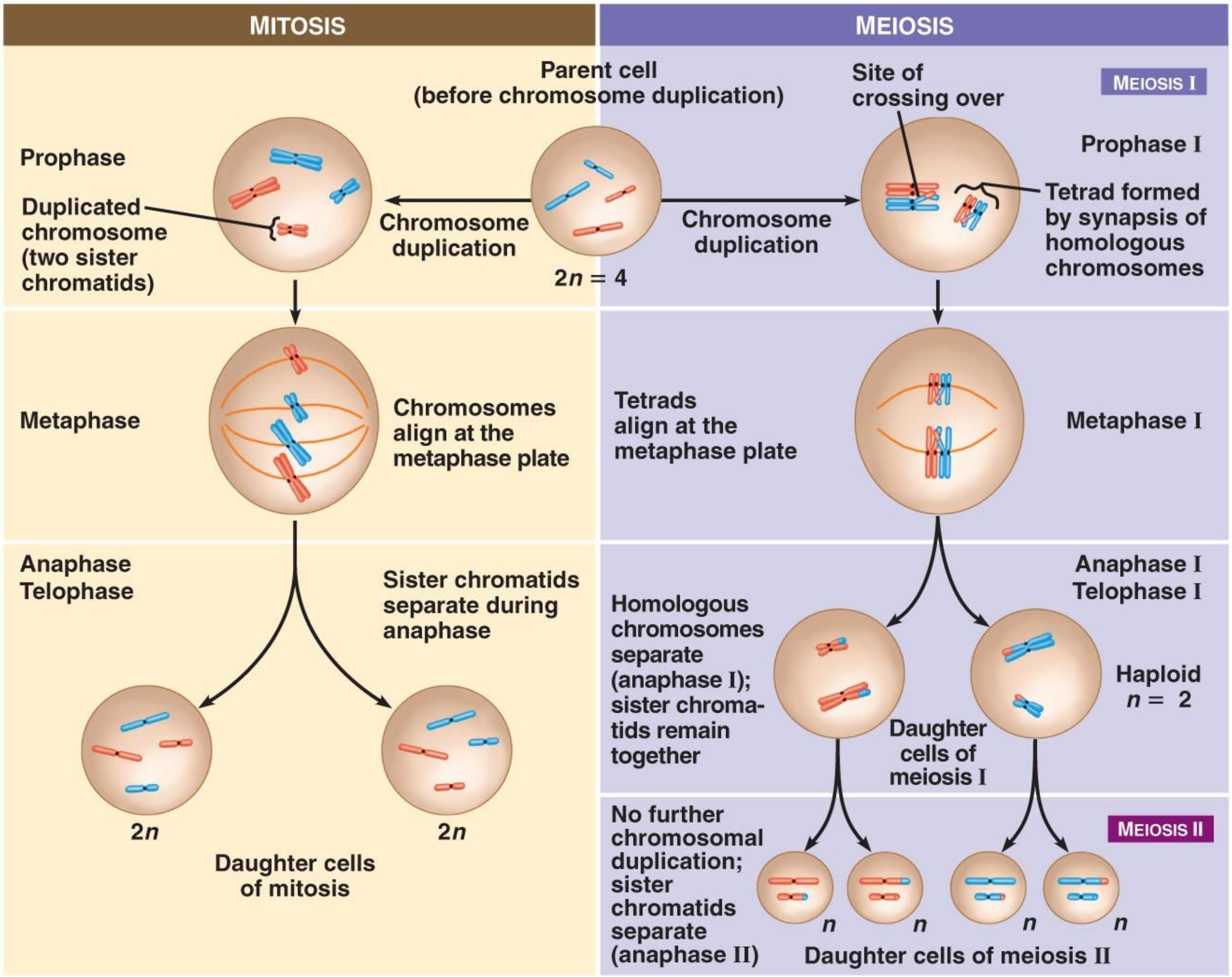


In early anaphase the centromeres split. Half the chromosomes move to one pole, half to the other pole.

In late anaphase the chromosomes have almost reached their respective poles. The cell membrane begins to pinch at the centre.

The cell membrane completes constriction in telophase. Nuclear membranes form around the separated chromosomes.

At mitosis completion, there are two cells with the same structures and number of chromosomes as the parent cell.



2.4| Meiosis Reduces the Chromosome Number from Diploid to Haploid in Germ Cells and Spores

- Unlike mitosis, meiosis reduces the amount of genetic material by one-half.
- Meiosis produces gametes or spores with only one haploid set of chromosomes.
- During sexual reproduction, gametes then combine through fertilization to reconstitute the diploid complement found in parental cells.
- If successfully completed, meiosis ensures genetic continuity from generation to generation. The process of sexual reproduction also ensures genetic variety among members of a species.
- A huge number of maternal-paternal chromosome combinations are possible at fertilization.
- **Crossing over;** results in genetic exchange between members of each homologous pair of chromosomes.

    - This process creates intact chromosomes that are mosaics of the maternal and paternal homologs from which they arise, further enhancing the potential genetic variation in gametes and the offspring derived from them.
    - Sexual reproduction reshuffles the genetic material, producing offspring that often differ greatly from either parent.

An Overview of Meiosis:

- ***First, how diploid cells give rise to haploid gametes or spores?***
    - Early in meiosis, homologous chromosomes form pairs; they synapse (or undergo synapsis)
    - Each synapsed structure, initially a bivalent, eventually gives rise to a tetrad consisting of four chromatids.
    - The presence of four chromatids demonstrates that both homologs (making up the bivalent) have duplicated.
    - So to achieve haploidy, two divisions are necessary.
        - The first division occurs in **meiosis I** and are described as **reductional division** (***because the # of centromeres, each representing one chromosome, is reduced by one-half***)
        - Components of each tetrad - representing the two homologs -separate, yielding two dyads.
        - Each dyad is composed of two sister chromatids joined at a common centromere.
        - The second division occurs during meiosis II and is described as an equational division (because the number of centromeres remains equal)
    - Exp. each dyad splits into two monads of one chromosome each. So, the two divisions potentially produce four haploid cells.
- **The First Meiotic Division: Prophase I**
    - Chromatin present in interphase thickens and coils into visible chromosomes.
    - Each chromosome is a double structure, held together by the molecular complex called cohesin.
    - Members of each homologous pair of chromosomes pair up, undergoing synapsis
    - Crossing over occurs between chromatids of synapsed homologs.
        - This stage is divided into five substages: **leptonema, zygonema, pachynema, diplonema, and diakinesis.**
        - **Leptonema:** the interphase chromatin material begins to condense, and the chromosome becomes visible (still extended tho).
            - Along each chromosome are chromomeres, localized condensations that resemble beads on a string.
            - Homology search (essential to the initial pairing of homologs) begins during leptonema.
        - **Zygonema:** the chromosome continue to shorten and thicken

            - During homolog search, H.chromosomes undergo initial alignment with one another → rough pairing completed by the end of zygonema.
            - The overall length of the lateral elements along the chromosome increases, and a more extensive ultrastructural component called the synaptonemal complex begins to form between the homologs.
            - Believed to be the vehicle for proper alignment during the pairing of homologs.
            - Upon completion of zygonema, the paired homologs are referred to as **bivalents**. They have replicated their DNA. # of bivalents in each species = haploid (n) number
        - **Pachynema;** during the transition, the chromosomes continue to coil and shorten, and further development of the synaptonemal complex occurs between two members of each bivalent.
        - Leads to synapsis, a more intimate pairing. → homologs now separated by only 100nm
            - Each homolog is now evident as a double structure.
            - Each bivalent contains four chromatids, whereas chromatids from maternal and paternal members of a homologous pair are called nonsister chromatids. Four-member structure; tetrad, contains two pairs of sister chromatids.
        - **Diplonema;** it is even more apparent that each tetrad consists of two pairs of sister chromatids.
            - Within each tetrad, each pair of sister chromatids begins to separate → one or more areas remain in contact where chromatids are intertwined; called **chiasma;** thought to represent a point where nonsister chromatids have undergone genetic exchange through the process of crossing over.
        - **Diakinesis;** the chromosomes pull farther apart, but nonsister chromatids remain loosely associated at the chiasmata.
            - The chiasmata move towards the ends of the tetrad as separation proceeds.
            - Terminalization begins in the late diplonema and is completed during diakinesis.
            - The nucleotide and nuclear envelope break down, and the two centromeres of each tetrad attach to the recently formed spindle fibers.
            - By completion of prophase I, the centromeres of each tetrad structure are present on the metaphase plate of the cell.
- **Metaphase, Anaphase, and Telophase I**
    - After prophase, metaphase I begins
    - **Metaphase I:**
        - The chromosomes have maximally shortened and thickened.

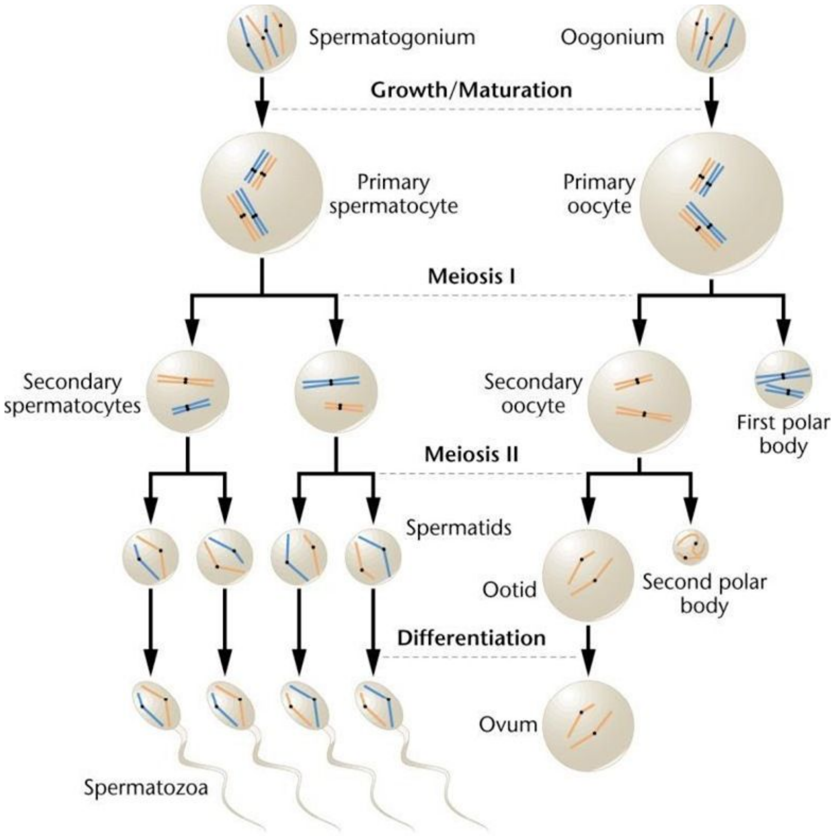
        - The terminal chiasmata of each tetrad are visible and appear to be the major factor holding the nonsister chromatids together.
        - Each tetrad interacts with spindle fibers, facilitating its movement to the metaphase plate.
        - The alignment of each tetra prior to the first anaphase is random; half to one pole, the other half to the other pole
        - A single centromeric region holds each pair of sister chromatids together → appears as a single unit.
        - Cohesion plays the major role in keeping sister chromatids together.
    - **Anaphase I;** cohesion is degraded between sister chromatids, except at the centromere region, which is protected by a shugoshin complex.
        - One-half of each tetrad (a dyad) is pulled toward each pole to the dividing cell.
        - Separation is the physical basis of **disjunction**, the separation of homologous chromosomes from one another. Nondisjunction is such a error.
        - If crossing over did not occur in meiosis I, each dyad at each pole would consist solely of either paternal or maternal chromatids.
    - **Telophase I;** reveals a nuclear membrane forming around the dyads.
        - The nucleus next enters into a short interphase period.
        - If interphase occurs, the chromosomes do not replicate because they already consist of two chromatids.
- **Second Meiotic Division: MEIOSIS II**
    - Is essential if each gamete or spore is to receive only one chromatid from each original tetrad.
    - **Prophase II;** each dyad is composed of one pair of sister chromatids attached by the common centromeric region.
    - **Metaphase II**; the centromeres are positioned on the equatorial plate.
        - When the shugoshin complex is degraded, the centromeres separate.
    - **Anaphase II;** initiated, the sister chromatids of each dyad are pulled to opposite poles
    - **Telophase II;** reveals one member of each pair of homologous chromosomes present at each pole, because the number of dyads is equal to the haploid number.
    - Each chromosome is now a monad.
    - Following cytokinesis in telophase II: four haploid gametes may result from a single meiotic event.

2.5| The Development of Gametes Varies in Spermatogenesis Compared to Oogenesis

*Spermatogenesis (male gametes) *Oogenesis (female gametes)

- **Spermatogenesis: takes place in the testes, the male reproductive organs:**

    - Begins with the enlargement of an undifferentiated diploid germ cell called a spermatogonium
    - This cell grows to become a primary spermatocyte, which undergoes the first meiotic division.
        - Products of this division are called secondary spermatocytes, and contain a haploid number of dyads
    - The secondary spermatocytes then undergo meiosis II
        - Each of these cells produces two haploid spermatids
    - Spermatids go through a series of developmental changes, spermiogenesis, to become highly specialized, motile spermatozoa, or sperm.
    - All sperm cells produced during spermatogenesis contain the haploid number of chromosomes and equal amounts of cytoplasm.
        - May be continuous or may occur periodically in mature male animals
- **Oogenesis: the formation of ova (sing.ovum) or eggs, occurs in ovaries, the female reproductive organs**
    - The daughter cells resulting from the two meiotic division of this process receive equal amounts of genetic material, but they do not receive equal amounts of cytoplasm
        - Almost all of the cytoplasm of the primary oocyte, itself derived from the oogonium, is concentrated in one of the two daughter cells.
        - The concentration of cytoplasm is necessary because a major function of the mature ovum is to nourish the developing embryo following fertilization
    - During anaphase I in oogenesis, the tetrads of the primary oocyte separate, and the dyads move toward opposite poles.
    - **During telophase I;** the dyads at one pole are pinched off with very little surrounding cytoplasm to form the first polar body.
        - First polar body may or may not divide again to produce two small haploid cells.
        - The other daughter cell produced by this first meiotic division contains most of the cytoplasm and is called the secondary oocyte.
    - The mature ovum will be produced from the secondary oocyte during the second meiotic division.
        - During this division, the cytoplasm of the secondary oocyte again divides unequally, producing an ootid and a second polar body
        - The ootid then differentiates into the mature ovum.
- Unlike the divisions of spermatogenesis, the two meiotic divisions of oogenesis may not be continuous.
    - In some animal species, the second division may directly follow the first.
    - In others, including humans, the first division of all oocytes begins in the embryonic ovary but arrests in prophase I.
    - The second division is completed only after fertilization.



2.6| Meiosis is Critical to Sexual Reproduction in All Diploid Organisms

- **Meiosis** is the mechanism by which the diploid amount of genetic information is reduced to the haploid amount. In animals, it leads to the formation of gametes, whereas in plants haploid spores are produced, which in turn lead to the formation of haploid gametes.
- Each diploid organism stores its genetic information in the form of homologous pairs of chromosomes.
    - Each pair consists of one member derived from the **maternal parent** and one from the **paternal parent**.
    - ***Following meiosis;*** haploid cells potentially contain either the paternal or the maternal representative of every homologous pair of chromosomes.
    - **Crossing over**; however (occurred in meiosis I) further reshuffles the alleles between the maternal and paternal members of each homologous pair, which then segregate and assort independently into gametes.
        - Results in genetic variation present in gametes
- **In many fungi;** the predominant stage of the life cycle consists of haploid vegetative cells.
    - They arise through meiosis and proliferate by mitotic cell division.
- In multicellular plants; the life cycle alternates between the diploid sporophyte stage and the haploid gametophyte stage.

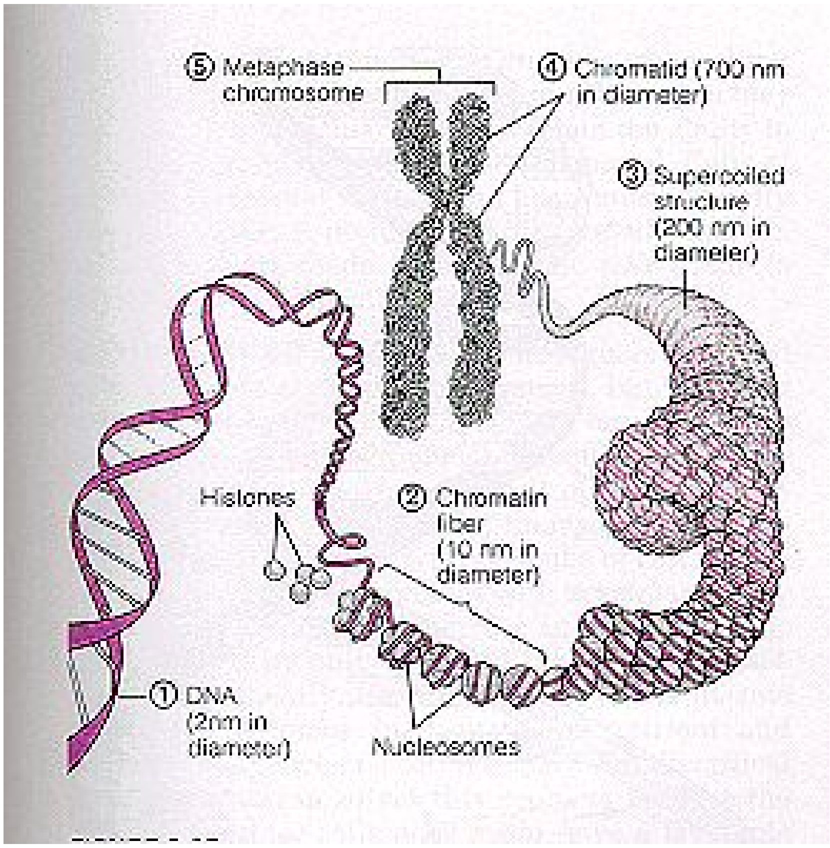
    - One or the other predominates in different plant groups during this “alternation of generations,” and the processes of meiosis and fertilization constitute the “bridges” between the sporophyte and gametophyte states.

Chapter 12: DNA ORGANIZATION IN CHROMOSOMES

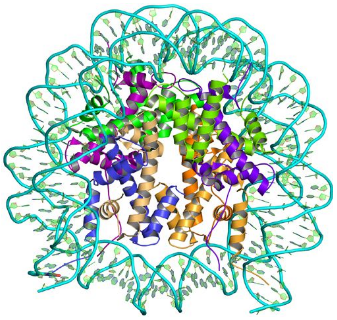
12.4| DNA Is Organized into Chromatin in Eukaryotes

- Most clearly visible as highly condensed structures during mitosis.
- However, after chromosome separation and cell division, cells enter the interphase stage of the cell cycle, at which time the components of the chromosome uncoil and de-condense into a form referred to as chromatin:
- **While in interphase;** the chromatin is dispersed throughout the nucleus.
- As the cell cycle progresses, cells may replicate their DNA and reenter mitosis, whereupon chromatin coils and condenses back into visible chromosomes once again.
    - This condensation represents a length contraction of some 10,000 times for each chromatin fiber.
- Due to the greater amount of DNA per chromosome in eukaryotes, as well as the presence of a large number of proteins associated with eukaryotic DNA.
- In a single human nucleus are 46 chromosomes containing sufficient DNA to extent almost 2 meters. Nucleus is about 5-10um in diameter.
- Although all cells carry a full genetic complement, different cells activate different sets of genes. Then, a highly ordered regulatory system must exist to govern the use of the genetic information.
    - Such a system must be in some way related to the molecular structure of the genetic material.
- **Chromatin Structure and Nucleosomes:**
    - Eukaryotic chromatin has a substantial amount of protein associated with the chromosomal DNA in all phases of the cell cycle.
    - The associated proteins can be categorized as either positively charged Histones or less positively charged Nonhistone proteins.
    - The histones play the most essential structural role:
        - They contain large amounts of the positively charged amino acids lysine and arginine, making it possible for them to bond electrostatically to the negatively charged phosphate groups of nucleotides.
        - Histones play an important role in chromatin structure
        - Chromatin produces regularly spaced diffraction rings, suggesting that repeating structural units occur along the chromatin axis.
        - If the histone molecules are chemically removed from chromatin, the regularity of this diffraction pattern is disrupted.
    - Digestion of chromatin by certain endonucleases (like micrococcal nuclease) yields DNA fragments that are approximately 200 base pairs in length or multiples therefor.

        - This enzymatic digestion is not random; if it were, we would expect a wide range of fragment sizes.
        - Chromatin consists of some type of repeating unit, each of which protects the DNA from enzymatic cleavage except where any two units are joined.
        - It is the area between the units that is attacked and cleaved by the endonuclease.
    - Electron microscopic observations of chromatin have revealed that chromatin fibers are composed of linear arrays of spherical particles.
        - The particles occur regularly along the axis of a chromatin strand and resemble beads on a string.
        - These particles (v-bodies) are now called nucleosomes.
        - Suggest the existence of repeating units.
    - Studies of the chemical association between histone molecules and DNA in the nucleosomes of chromatin show that histones H2A, H2B, H3, and H4 occur as two types of tetramers, (type 1 and type (2))-H2A)2
        - Each repeating nucleosome unit consists of one of each tetramer i association with about 200 base pairs of DNA.
        - Explains the interaction of histones and DNA in chromatin.
    - When the nuclease digestion time is extended, some of the 200 base pairs of DNA are removed from the nucleosome, creating a nucleosome core particle consisting of 147 base pairs.
        - The DNA lost in the prolonged digestion is responsible for linking nucleosomes together.
        - This linker DNA is associated with the fifth histone, H1.
- A 147-bp length of the 2-nm-diameter DNA molecule coils around an octamer of histones in a left-handed superhelix that completes about 1.7 turns per nucleosome.
    - Each nucleosome, ellipsoidal in shape, measures about 11 nm at its longest point.
    - The formation of the nucleosome represents the first level of packing, whereby the DNA helix is reduced to about one-third of its original length by winding around the histones.
        - In the nucleus, the chromatin fiber is further packed into a thicker, 30-nm-diameter structure that was initially called a solenoid.
        - This structure (dependent on the presence of histone H1) consists of numerous nucleosomes coiled around and stacked upon one another, creating a second level of packing → creating a sixfold increase in compaction of the DNA.
        - It is this structure that is characteristic of an uncoiled chromatin fiber in interphase of the cell cycle.
        - It is further compacted into a series of looped domains, which further condense the chromatin fiber into a structure that is 300 nm in diameter.

        - These coiled chromatin fibers are compacted into the chromosome arms that constitute a chromatid, one of the longitudinal subunits of the metaphase chromosome.
    - Haploid genome contains more than 3 billion base pairs of DNA distributed among 23 chromosomes.
    - The diploid cell contains twice that amount (46 chromosomes)
    - A packing ratio (the ratio of DNA length to the length of the structure containing it) of about 500 to 1 must be achieved.
- 

- **Chromatin Remodeling**
    - When present in several levels of compaction within the chromatin fiber, DNA is inaccessible to interaction with other important DNA-binding proteins.
    - To accommodate these protein-DNA interactions, chromatin must be induced to change its structure, a process referred to as; chromatin remodeling.
    - To allow replication and gene expression, chromatin must relax its compact structure and expose regions of DNA to these proteins, and there must also be a mechanism for reversing the process during periods of inactivity.



- Double-helical ribbon represents 147 bp of DNA surrounding four pairs of histone proteins. This configuration is repeated over and over in the chromatin fiber and is the principal packaging unit of DNA in the eukaryotic nucleus.

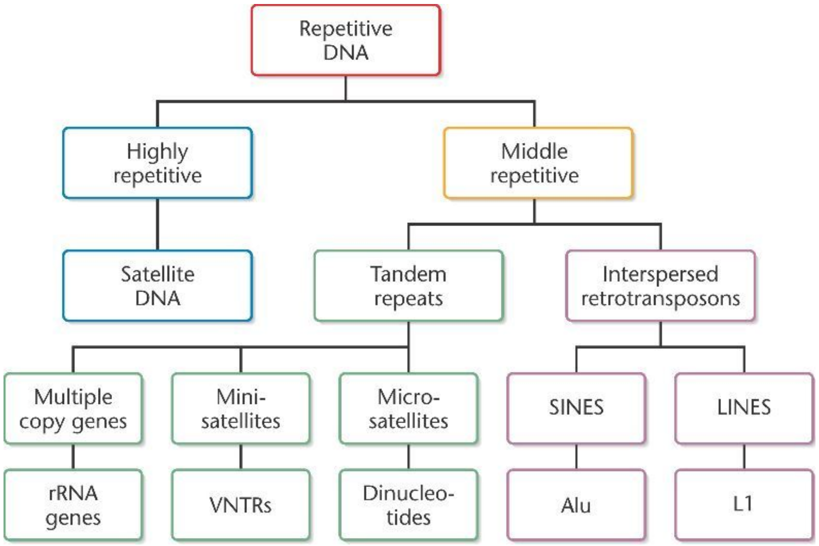
- Unstructured histone tails that are not packed into the folded histone domains within the core of the nucleosomes but instead protrude from it.
    - These histone tails although provide potential targets along the chromatin fiber for a variety of chemical modifications that may be linked to genetic functions, including chromatin remodeling and the possible regulation of gene expression.
    - Acetylation is a histone modification; by the action of the enzyme histone acetyltransferase (HAT).
        - The addition of an acetyl group to the positively charged amino group present on the side chain of the amino acid lysine effectively changes the net charge of the protein by neutralizing the positive charge.
        - Lysine is in abundance in histones and acetylation is linked to gene activation.
        - High levels of acetylation open up, or remodel, the chromatin fiber, increasing the regions of active genes and decreasing in inactive regions.
    - **Methylation and phosphorylation of amino acids;** result from the action of enzymes called methyltransferases and kinases, respectively.
    - **Methyl groups** can be added to both arginine and lysine residues in histones and this change has been correlated with gene activity.
    - Phosphate groups can be added to the hydroxyl groups of the amino acids serine and histidine, introducing a negative charge on the protein.
        - During the cell cycle, increased phosphorylation, of histone H3, is known to occur at characteristic times
        - It is believed to be related to the cycle of chromatin unfolding and condensation that occurs during and after DNA replication.
    - All these chemical modifications are reversible, under the direction of specific enzymes.
    - Methylation of the nitrogenous base cytosine within polynucleotide chains of DNA, forming 5-methylcytosine, is usually negatively correlated with gene activity.
        - Methylation of cytosine occurs most often when the nucleotide cytidylic acid is next to the nucleotide guanylic acid, forming a CpG island.
        - Methylation can then have BOTH negative and positive impacts on gene activity.
    - **Epigenetics**; the study of modifications of an organism's genetic and phenotypic expression that are not attributable to alteration of the DNA sequence making up a gene.
- **Heterochromatin**
    - **Euchromatin and heterochromatin;** the parts of chromosomes that are uncoiled and those that remain condensed, respectively - b/c some parts of the chromosome remain condensed and stain deeply during interphase, while most parts are uncoiled and do not stain.
    - **Heterochromatic** areas are genetically inactive because they either lack genes or contain genes that are repressed.

        - Heterochromatin replicates later during the S phase of the cell cycle than does euchromatin
        - Discovery of this provided the first clues that parts of eukaryotic chromosomes do not always encode proteins.
        - Telomere maintains chromosome structural integrity. Centromere is involved in chromosome movement during cell division.
        - In some cases, whole chromosomes are heterochromatic.
    - Exp. mammalian Y chromosome, genetically inert.
        - The inactivated X chromosome in mammalian females is condensed into an inert heterochromatic Barr body.
    - When certain heterochromatic areas from one chromosome are translocated to a new site on the same or another nonhomologous chromosome, genetically active areas sometimes become genetically inert if they now lie adjacent to the translocated heterochromatin.
        - **Position effect** - this existing euchromatin; the position of a gene or group of genes relative to all other genetic material may affect their expression.

12.5| Chromosome Banding Differentiates Regions along the Mitotic Chromosome

- **Chromosomal banding techniques;** cytological procedures that make it possible to differentially stain along the longitudinal axis of mitotic chromosomes → the staining patterns resemble the bands of polytene chromosomes.
- **C-banding:** identifies a specific area of the chromosome composed of heterochromatin (treated denatured chromosomes with Giemsa stain, and only the centromeric regions of mitotic chromosomes take up the stain)
- **G-bands;** involved the digestion of the mitotic chromosomes with the proteolytic enzyme trypsin, followed by giemsa staining.
    - This method produces a staining pattern differentially along the length of each chromosome.
- Bands play an important role in cytogenetic analysis, particularly in humans.
    - Homologs can be distinguished from one another, and when a segment of one chromosome has been translocated to another chromosome, its origin can be determined with great precision.

12.6| Eukaryotic Genomes Demonstrate Complex Sequence Organization Characterized by Repetitive DNA



- **Repetitive DNA**
- **Multiple-copy genes; functional genes that are present in more than one copy and so are repetitive in nature.**
    - The majority of repetitive sequences do not encode proteins, many are still transcribed and the resultant RNAs play multiple roles in eukaryotes, including chromatin remodelling.
    - Three main categories of repetitive sequences; (1) **heterochromatin** found to be associated with centromeres and make up telomeres; (2) **tandem repeats** of both short and long DNA sequences; and (3) **transposable sequences** that are interspersed throughout the genome of eukaryotes.
- **Satellite DNA:**
    - Nucleotide composition (eg...% of G=C v.s A=T pairs) of the DNA of a particular species is reflected in the DNA's density, which can be measured with a technique called Sedimentation Equilibrium Centrifugation → which determines the molecule's density.
    - When eukaryotic DNA is analyzed in this way; the majority is present and represented as a single main band, of fairly uniform density.
        - One or more additional peaks although indicate the presence of DNA that differs slightly in density.
        - This component (Satellite DNA); makes up a variable proportion of the total DNA, depending on the species.
            - Prokaryotes do not contain satellite DNA
        - Certain portions of DNA reannealed more rapidly than others, so rapid reannealing is characteristic of multiple DNA fragments composed of identical or nearly identical nucleotide sequences-the basis for "repetitive DNA"
        - Satellite DNA is highly repetitive DNA, consisting of short sequences repeated a large number of times.

        - These sequences are present at tandem (adjacent) repeats clustered in very specific chromosomal areas known to be heterochromatic--the regions flanking centromeres.
    - **In situ hybridization;** involves molecular hybridization between an isolated fraction of labeled DNA or RNA probes and the DNA contained in the chromosomes of a cytological preparation.
    - Following hybridization, autoradiography was performed to locate the chromosome areas complementary to the fraction of DNA or RNA.
        - Radioactive molecular probes hybridize with DNA of centromeric regions of mouse mitotic chromosomes.
        - Satellite DNA differs from main-band DNA in its molecular composition.
        - It is composed of short repetitive sequences.
        - It is found in the heterochromatic centromeric regions of chromosomes.
- **Centromeric DNA Sequences:**
    - Separation of homologs (during mitosis and meiosis) depends on centromeres; the primary constrictions along eukaryotic chromosomes
    - The minimal region of the centromere that supports the function of chromosomal segregation is designated the CEN region.
        - Within this heterochromatic region, the DNA binds a platform of proteins, which in multicellular organisms includes the kinetochore that binds to the microtubules making up the spindle fiber during division.
        - Each centromere serves an identical function, CENs from different chromosomes are remarkably similar in their DNA SEQUENCES.
        - Portions near the 3’ end of this DNA region are most critical to centromere function since mutations in them, but not those nearer the 5’ end, disrupt centromere function.
            - The DNA of this region appears to be essential to the eventual binding to the spindle fiber.
    - **Alphoid family;** in humans, found mainly in the centromere regions.
        - Each about 170 bp in length, are present in tandem arrays of up to 1 million base pairs.
        - The H3 histone (normal part of most euK. nucleosomes) is substituted by a variant histone designated CENP-A in centromeric heterochromatin.
        - The unique N-terminal protein tails that make CEN-P unique are involved in the binding of kinetochore proteins that are essential to the microtubules of spindle fibers.
- **Middle Repetitive Sequences: VNTRs and STRs**
    - Middle (moderately) repetitive DNA, recognized by reassociation kinetic studies.
    - Although it does not include some duplicated genes (i.e those encoding rRNA), most prominent in this category are either noncoding tandemly repeated sequences or interspersed sequences. → no function found.
    - Variable number tandem repeats (VNTRs); may be 15-100 bp long and are found within and between genes.

    - **Minisatellites;** such clusters dispersed throughout the genome of VNTRs
    - Di-tri-tetra and pentanucleotides (microsatellites or short tandem repeats-STRs)
        - They are dispersed throughout the genome and vary among individuals in the number of repeats present at any site.
- **Repetitive Transposed Sequences: SINEs (exp. Alu family) and LINEs (exp. L1 family)**
    - Sequences that are interspersed individually throughout the genome, rather than being tandemly repeated. Short or long and many have the added (transposable sequences; are mobile and can potentially move to different locations within the genome.
- **Middle Repetitive Multiple-Copy Genes**
    - In some cases, middle repetitive DNA includes functional genes present tandemly in multiple copies.

12.7| The Vast Majority of a Eukaryotic Genome Does Not Encode Functional Genes

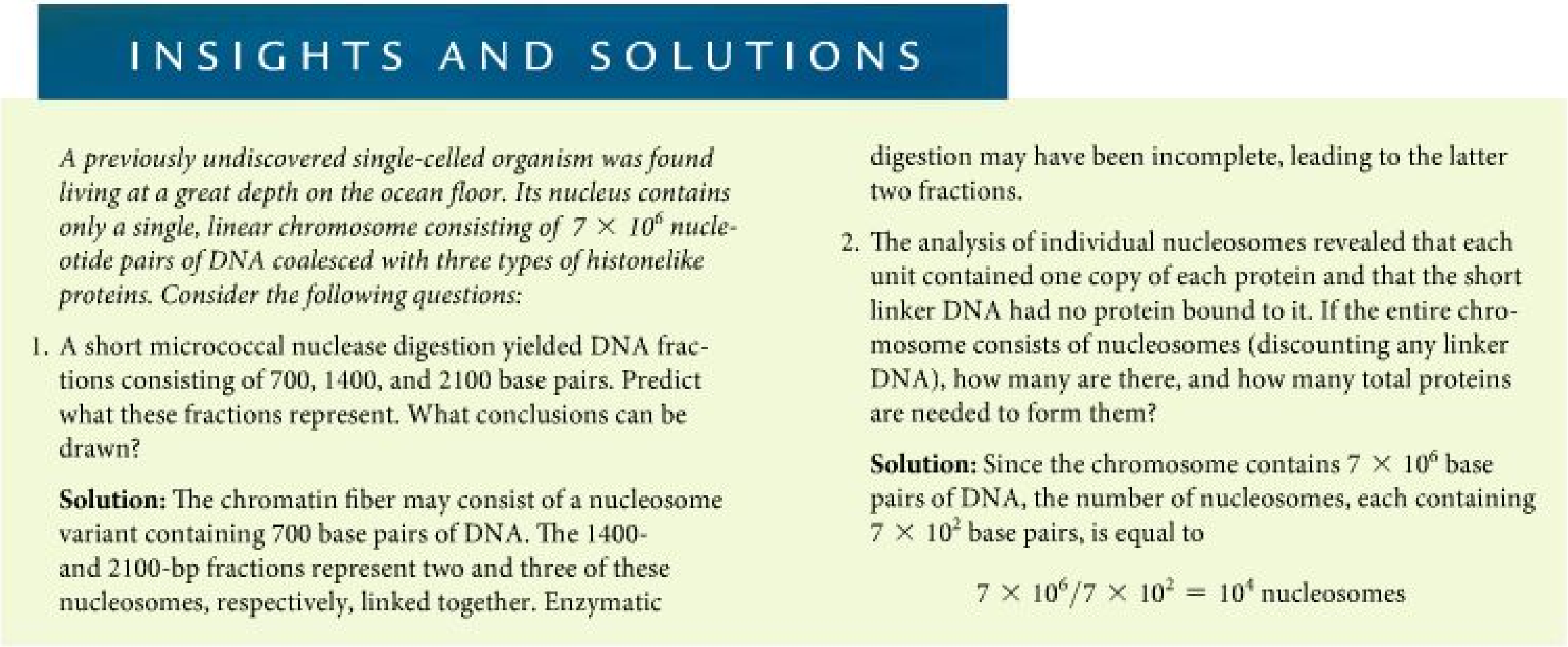
- Approximately 50% of all DNA sequences comprises various forms of highly repetitive and moderately repetitive DNA.
- A large amount of the DNA consists of single-copy sequences that appear to be noncoding.
- **Pseudogenes;** DNA sequences representing evolutionary vestiges of duplicated copies of genes that have undergone significant mutational alteration.
    - As a result, although they show some homology to their parent gene, they are usually not transcribed because of insertions and deletions throughout their structure.
- Only a very small part of the genome actually codes for proteins thought!! LOL wuut? All this time? Bruh.
- In humans, the coding regions of the estimated 20,000 functional genes occupy only about 2% of the total DNA sequence making up the genome.

CHAPTER SUMMARY:

1. Bacteriophage and bacterial chromosomes, in contrast to euK, are largely devoid of associated proteins, are of much smaller zine and most consist of circular DNA.
2. Eukaryotic chromatin is a nucleoprotein organized into repeating units called nucleosomes (composed of 200 base pairs of DNA, an octamer of four types of histones, plus one linker histone)
3. Nucleosomes provide a mechanism for compaction of chromatin within the nucleus.
    a. Several forms of chemical modification, 4 exp. Acetylation and methylation, may alter the level of compaction; chromatin remodelling → is critical to replication and transcription of DNA
4. Heterochromatin, prematurely condensed in interphase and for the most part genetically inert, is illustrated by centromeric and telomeric regions of eukaryotic chromosomes, the Y chromosome and Barr Body.

5. Chromosome banding techniques provide a way to subdivide and identify specific regions of mitotic chromosomes.
6. Eukaryotic genomes demonstrate complex sequence organization characterized by numerous categories of repetitive DNA, consisting of either tandem repeats clustered in various regions of the genome or single sequences repeatedly interspersed at random in the genome.
7. The vast majority of the DNA in most euK. Genomes does not encode functional genes.

Chapter Practice Questions:



INSIGHTS AND SOLUTIONS

A previously undiscovered single-celled organism was found living at a great depth on the ocean floor. Its nucleus contains only a single, linear chromosome consisting of 7×10^6 nucleotide pairs of DNA coalesced with three types of histonelike proteins. Consider the following questions:

1. A short micrococcal nuclease digestion yielded DNA fractions consisting of 700, 1400, and 2100 base pairs. Predict what these fractions represent. What conclusions can be drawn?

Solution: The chromatin fiber may consist of a nucleosome variant containing 700 base pairs of DNA. The 1400- and 2100-bp fractions represent two and three of these nucleosomes, respectively, linked together. Enzymatic digestion may have been incomplete, leading to the latter two fractions.

2. The analysis of individual nucleosomes revealed that each unit contained one copy of each protein and that the short linker DNA had no protein bound to it. If the entire chromosome consists of nucleosomes (discounting any linker DNA), how many are there, and how many total proteins are needed to form them?

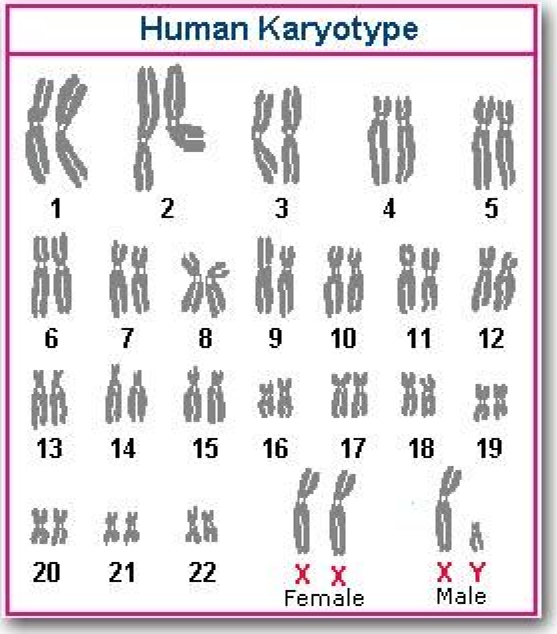
Solution: Since the chromosome contains 7×10^6 base pairs of DNA, the number of nucleosomes, each containing 7×10^2 base pairs, is equal to

$$7 \times 10^6 / 7 \times 10^2 = 10^4 \text{ nucleosomes}$$

Chapter 7: WHAT ARE THE MECHANISMS OF SEX DETERMINATION?

7.3| The Y Chromosome Determines Maleness in Humans

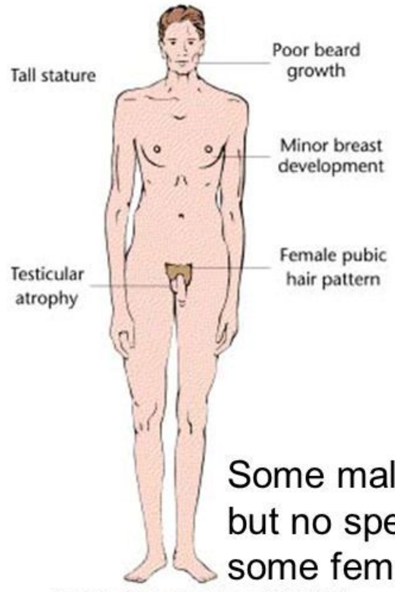
- 46 is the human diploid number of chromosomes present.
- Of the normal 23 pairs of human chromosomes, one pair was shown to vary in configuration in males and females.
    - X and Y chromosome.
    - The human female has two X (XX) chromosomes
    - The human male has one X and one Y chromosome (XY)
- The Y chromosome does determine maleness in humans (the presence of this Y chromosome indicates maleness - is sex determining)
- Traditional human karyotypes from a normal male and female
    - Each contain 22 pairs of autosomes and two sex chromosomes - females being XX and males being XY.



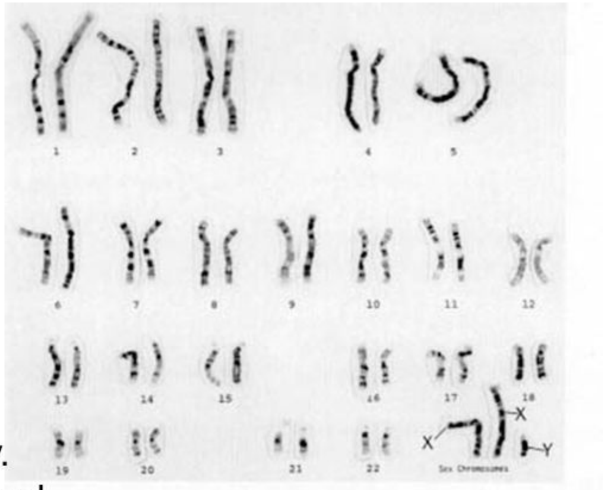
- **Klinefelter and Turner Syndromes**
    - These two human abnormalities are characterized by aberrant sexual development
    - **Felter syndrome (47, XXY)**
        - Infected individuals are generally tall and have long arms and legs and large hands and feet.

        - They usually have genitalia and internal ducts that are male, but their testes are rudimentary and fail to produce sperm.
        - Feminine sexual development is not entirely suppressed. Slight enlargement of the breasts (gynecomastia) is common, and the hips are often rounded.
        - This ambiguous sexual development, intersexuality, can lead to abnormal social development. Intelligence is often below the normal range.
        - Individuals have more than one X chromosome. Most often, they have an XXY complement in addition to 44 autosomes, which is why people with this karyotype are designated, 47,XXY.
    - **Turner syndrome (45, X)**
        - Infected individual has female external genitalia and internal ducts, but the ovaries are rudimentary (also, short stature - 5ft. Cognitive impairment, skin folds on the back of the neck, and underdeveloped breasts)
        - Individuals most often have only 45 chromosomes, including just a single X chromosome; thus, they are designated 45, X.
        - In the absence of a Y chromosome, no masculinization occurs.
        - We cannot conclude anything regarding sex determination under circumstances where a Y chromosome is present without an X
            - Because Y-containing human embryos lacking an X chromosome (45, Y) **do not survive.**
    - Both conditions result from nondisjunction, the failure of the X chromosomes to segregate properly during meiosis
    - These conditions have led to the conclusion that the Y chromosome determines maleness and thus is the basis for phenotypic sex determination in humans.
        - In its absence, the person's sex is female, even if only a single X chromosome is present.
    - Klinefelter occurs in about 1/660 male births
        - Manifestations are often more severe in individuals with a greater number of X chromosomes (exp. 48,XXXY **>** 48,XXYY **<** 48, XXXXY) even though the karyotypes are similar phenotypically to 47,XXY
    - Turner can also result from individuals with karyotypes other than 45,X, including individuals called mosaics; whose somatic cells display two different genetic cell lines, each exhibiting a different karyotype.
        - Such cell lines result from a mitotic error during early development, the most common chromosome combinations being 45,X/46,XY and 45,X/46,XX.
        - An embryo that began life with a normal karyotype can give rise to an individual whose cells show a mixture of karyotypes and who exhibits varying aspects of this syndrome (not inherent but can be derived)
        - Occurs in 1/2000 female births, lower than klinefelter b/c the majority of 45,X fetuses die in utero and are aborted spontaneously.

Klinefelter Syndrome (47, XXY)



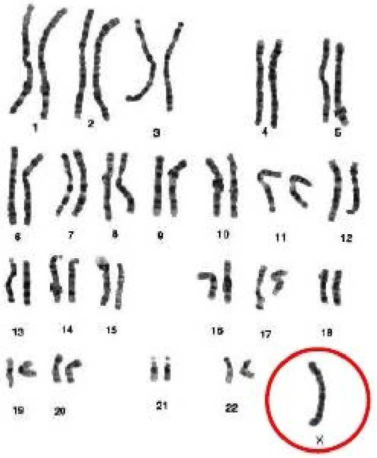
(a) Klinefelter Syndrome (47,XXY)

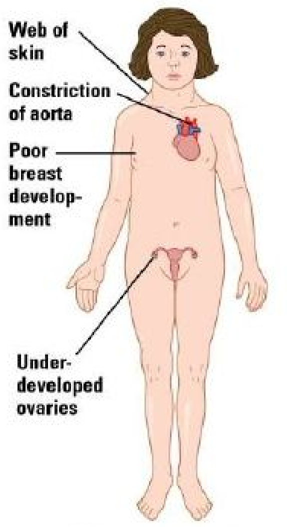


Some male dev.
but no sperm, and
some female dev. too.

2 in 1000 male births

Turner's Syndrome





(b) A woman with Turner syndrome (XO)

- Female with only one X chromosome (XO)
- Sterile

- **47, XXX Syndrome**
    - The presence of three X chromosomes along a normal set of autosomes results in female differentiation.
    - **triplo-X** is a highly variable syndrome that accompanies this genotype and occurs in about 1/1000 female births.
    - Women with 47,XXX are frequently normal and may remain unaware of their abnormality in chromosome number unless a karyotype is done.
        - In other cases, underdeveloped secondary sex characteristics, sterility, delayed development of language and motor skills, and mental retardation may occur.
        - In many cases, the presence of additional X chromosomes appears to disrupt the delicate balance of genetic information essential to normal female development.
- 47,XXY Condition
    - The only deviation from diploidy is the presence of an additional Y chromosome in an otherwise normal male karyotype.
    - Infected individuals are significantly above average in height, have subnormal intelligence, and all suffer personality disorders.
    - Above-average height (over 6ft.) and subnormal intelligence have been generally substantiated and the frequency of males displaying this karyotype is higher among people in penal and mental institutions among nonincarcerated populations.
        - The only correlation between nonincarcerated populations displayed by XXY males is that such individuals are over 6ft tall.
        - Many XYY males are present in the population who do not exhibit antisocial behavior and who lead normal lives.
        - There is then a high, but not constant, correlation between the extra Y chromosome and the predisposition of these males to exhibit behavioral problems.
- **Sexual Differentiation in Humans**
    - **During early development,** every human embryo undergoes a period when it is potentially hermaphroditic.
    - **By the fifth week of gestation**, gonadal primordia (the tissues that will form the gonad) arise as a pair of gonadal (genital) ridges associated with each embryonic kidney.
        - The embryo is potentially hermaphroditic because at this stage its gonadal phenotype is sexually indifferent-male or female reproductive structures cannot be distinguished, and the gonadal ridge tissue can develop to form male or female gonads.
        - As development progresses, primordial germ cells migrate to these ridges, where an outer cortex and inner medulla form (cortex and medulla are the outer and inner tissues of an organ, respectively)

        - The cortex is capable of developing into an ovary, while the medulla may develop into a testis.
        - Two sets of undifferentiated ducts called the Wolffian and Mullerian ducts exist in each embryo.
        - Wolffian ducts differentiate into other organs of the male reproductive tract
        - Mullerian ducts differentiate into structures of the female reproductive tract.
    - **Bipotential gonads**; because gonadal ridges can form either ovaries or testes ← the common name.
    - What triggers gonadal ridge development into testes or ovaries?
        - The presence or absence of a Y chromosome is the key.
        - If the cells of the ridge have an XY constitution, development of the medulla into a testis is initiated around the seventh week.
        - In the absence of the Y chromosome, no male development occurs, the cortex of the ridge subsequently forms ovarian tissue, and the Mullerian duct forms oviducts, uterus, cervix, and portions of the vagina.
        - (depending on which pathway is initiated), parallel development of the appropriate male or female duct system then occurs, and the other duct system degenerates (what is not used for development)
        - Presence of a Y chromosome and development of the testes although also inhibit formation of female reproductive organs.
    - **In females,** (as the 12th week of fetal development approaches) oogonia within the ovaries begin meiosis, and primary oocytes can be detected
        - By the 25th week of gestation, all oocytes become arrested in meiosis and remain dormant until puberty is reached some 10-15 years later.
    - **In males,** primary spermatocytes are not produced until puberty is reached.
- **The Y Chromosome and Male Development**
    - The Y chromosome has about 50 genes compared to about 1000 genes on the X.
    - The X and Y chromosomes are said to have originated from a homologous pair of autosomes about 200 million years ago. Yet they share a list of sequence similarities
    - Research reveals that some have homologous counterparts on the X chromosome and others do not.
    - Present on both ends of the Y chromosome are **pseudoautosomal regions (PARs)**
        - These share homology with regions on the X chromosome and synapse and recombine with it during meiosis.
        - The presence of such pairing is important to segregation of the X and Y chromosomes during male gametogenesis.
        - The remainder of the chromosomes-body(~95%), does not synapse or recombine with the X chromosome.

    - As a result; it was originally referred to as the nonrecombining region of the Y (NRY). → this region is the **male-specific region of the Y (MSY)**
    - Some regions of the MSY share homology with genes on the X chromosome and others do not.
  - The MSY is divided about equally between euchromatic regions, containing functional genes, and heterochromatic regions; lacking genes.
    - Within euchromatin; adjacent to the PAR of the short arm of the Y chromosome, is a critical gene that controls male sexual development, called the **Sex-determining region Y (SRY)**
    - In humans, the absence of a Y chromosome almost always leads to female development, thus, this gene is absent from the X chromosome.
  - At 6-8 weeks of development, the SRY gene becomes active in XY embryos.
    - SRY encodes a protein that causes the undifferentiated gonadal tissue of the embryo to form testes.
    - This protein is called the **testis-determining factor (TDF)**
    - SRY (or closely related version) is present in all mammals
  - In chickens, a similar modification of the gene exists **(DMRT1)** and is located on the Z chromosome.
  - The ability to identify the presence or absence of DNA sequences in rare individuals whose sex-chromosome composition does not correspond to their sexual phenotype has provided evidence that **SRY is the gene responsible for male sex determination.**
    - There are human males with (XX) and no Y chromosome. Often, attached to one of their X chromosomes is the region of the Y that contains SRY.
    - There are females who have one X and one Y (XY) chromosome. Their Y is almost always missing the SRY gene.
  - *When mouse DNA containing Sry is injected into normal XX mouse eggs, most of the offspring develop into males:
    - TDF(testes-determining factor) functions as a transcription factor, a DNA-binding protein that interacts directly with regulatory sequences of other genes to stimulate their expression.
    - While TDF behaves as a master switch that controls other genes downstream in the process of sexual differentiation, identifying TDF target genes has been difficult.
    - Another potential target for activation by TDF is the gene for Mullerian inhibiting substance (MIS - or Mullerian inhibiting hormone, MIH, or anti-Mullerian hormone)
    - Cells of the developing testes secrete MIS.
    - MIS protein causes regression (atrophy) of cells in the Mullerian duct.
    - Degeneration of the duct prevents formation of the female reproductive tract.

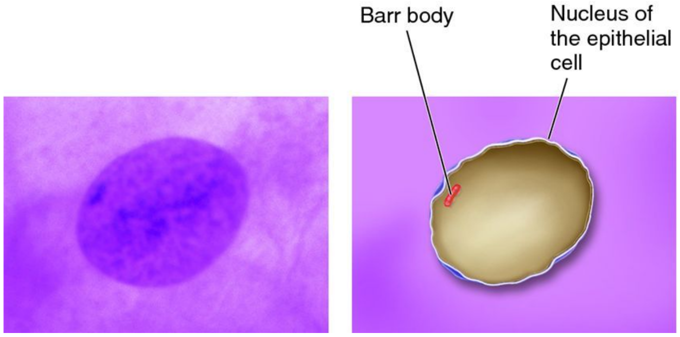
- Human SOX9 gene, when activated by SRY, leads to the differentiation of cells that form the seminiferous tubules that contain male germ cells (blocks testes development and signs of ovarian development occur.
- SF1 is involved in the regulation of enzymes affecting steroid metabolism (active in both males and female bisexual genital ridge, persisting until the point in development when testis formation is apparent)
    - At that time, its expression persists in males but is extinguished in females.
    - In mice, testicular development may be actively repressed throughout the life of females by downregulating expression of specific genes. (deletion of FOXL2 in adult female mice (which encodes a transcription factor) leads to transdifferentiation of the ovary into the testis)
- The MSY consists of about 23 million base pairs and can be divided into three regions
    - **The first region** is the **X-transposed region**. It comprises about 15 percent of the MSY and was originally derived from the X chromosome in the course of human development.
        - The X-transposed region is 99% identical to region Xq21 of the modern human X chromosome.
        - Two genes, both with X chromosome homologs, are present in this region.
    - Sequences called palindromes-sequences of base pairs that read the same but in the opposite direction on complementary strands-are present throughout the MSY.
    - Recombination between palindromes on sister chromatids of the Y during replication is a mechanism used to repair mutations in the Y.
- The MSY of the human Y chromosome is very different in sequence structure than the MSY from chimpanzees.
    - So rapid evolution has occurred since separation of these species over 6 million years ago
    - Over 30% of the chimpanzee mSY sequence has no homologous sequence in the human MSY.
    - The chimpanzee MSY has lost many protein-coding genes compared to common ancestors but contains twice the number of palindromic sequences as the human MSY.
    - **The second area** of the MSY is designated the **X-degenerative region,** comprising about 20% of MSY.
        - Contains DNA sequences that are even more distantly related to those present on the X chromosome.
        - It contains 27 single-copy genes and a number of pseudogenes (genes whose sequences have degenerated sufficiently during evolution to render them nonfunctional)

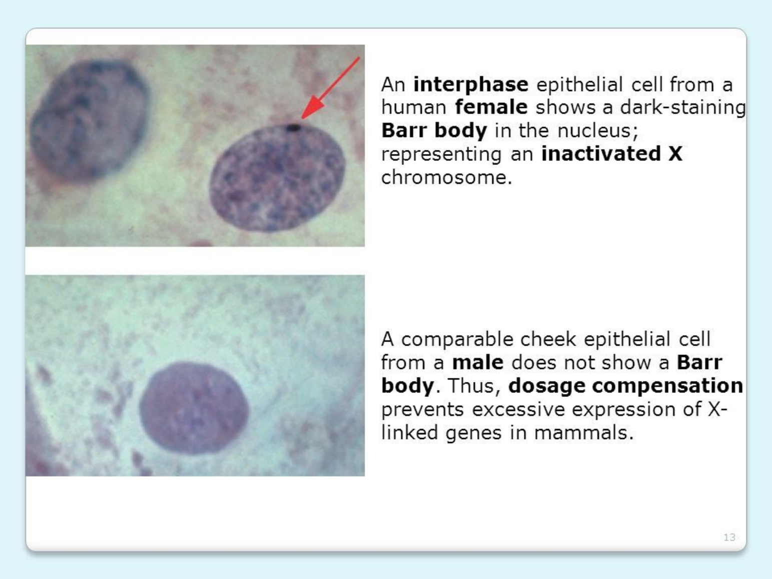
            - All genes share some homology with counterparts on the X chromosome (one of which is the SRY gene)
            - Other X-degenerative genes that encode protein products are expressed ubiquitously in all tissues in the body, but SRY is expressed only in the testes.
        - The third area; **the ampliconic region,** contains about 30% of the MSY, including most of the genes closely associated with the development of testes.
            - These genes lack counterparts on the X chromosome, and their expression is limited to the testes.
            - There are 60 transcription units (genes that yield a product) divided among nine gene families in this region, most represented by multiple copies.
            - Members of each family have nearly identical (>98%) DNA sequences.
            - Each repeat unit is an amplicon and is contained within seven segments scattered across the euchromatic regions of both the short and long arms of the Y chromosome.
            - Genes in this region encode proteins specific to the development and function of the testes, and the products of many of these genes are directly related to fertility in males.
    - Advanced paternal age is associated with an increased risk in offspring of congenital disorders with a genetic basis, including certain cancers, schizophrenia, autism, and other conditions, known as "paternal age effects (PAE)"
        - There is the presence of specific PAE mutations including numerous ones on the Y chromosome.
        - PAE mutations are positively selected for and result in an enrichment of mutant sperm over time.

7.5| Dosage Compensation Prevents Excession Expression of X-linked Genes in Mammals.

- **"Genetic dosage"** difference between males and females with attendant problems, for all X-linked genes → the disparity between XY chromosomes and equal numbers of autosomes present in the cells of both sexes.
    - The additional X chromosome in both males and females are thought to compound this dosage problem.
    - Embryonic development depends on proper timing and precisely regulated levels of gene expression. Otherwise, disease phenotypes or embryonic lethality can occur.
- **Barr Bodies**

- **Sex chromatin body/ Barr Body;** an activated X-chromosome present in a female somatic cell
    - Darkly staining body in interphase nerve cells of females that is absent in males.
    - This body can be easily demonstrated in female cells, derived from the buccal mucosa (cheek cells) or in fibroblasts (undifferentiated connective tissue cells) but not in similar males.
    - Highly condensed, about 1um in diameter, lying against the nuclear envelope of interphase cells and stains positively in the Feulgen reaction, a cytochemical test for DNA.
    - Regardless of how many X chromosomes a somatic cell possesses, all but one of them appear to be inactivated and is the Barr bodies.
    - The number of Barr bodies follows an N-1 rule, where N is the total number of X chromosomes present.
- Also, about 15% of the human X-chromosomal genes actually escape inactivation. Clearly, not every gene on the X requires inactivation.
    - In other cases, excessive expression of certain X-linked genes might still occur at critical times during development despite apparent inactivation of superfluous X chromosomes.





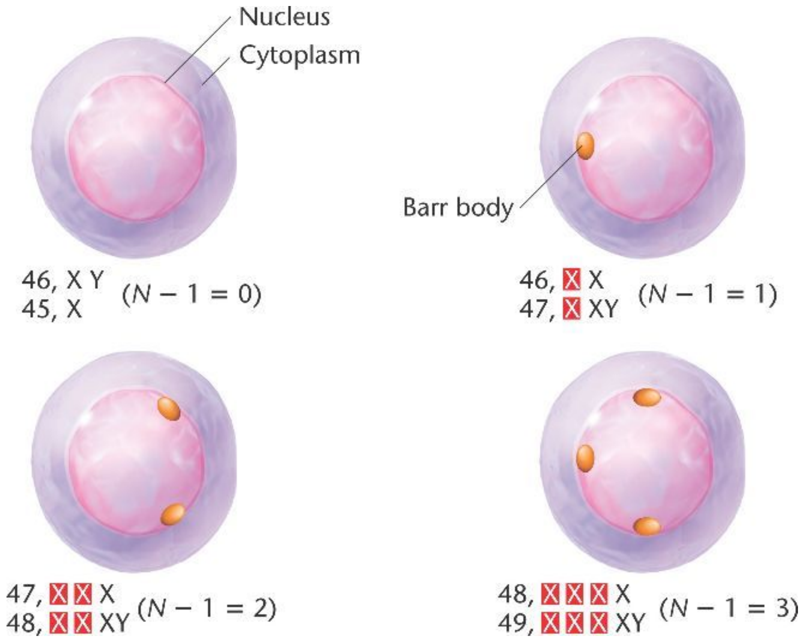
An **interphase** epithelial cell from a human **female** shows a dark-staining **Barr body** in the nucleus; representing an **inactivated X** chromosome.

A comparable cheek epithelial cell from a **male** does not show a **Barr body**. Thus, **dosage compensation** prevents excessive expression of X-linked genes in mammals.

Calculating # of Barr Bodies: N rule:

Barr Bodies = **N-1** (where **N** is the **# of chromosomes** present in the cell)

- **The Lyon Hypothesis**
    - The inactivation of X chromosomes occurs randomly in somatic cells at a point early in embryonic development, most likely sometime during the blastocyst stage of development
    - Once inactivation occurred, all descendant cells have the same X chromosome inactivated as their initial progenitor cell.
    - This hypothesis is based on observations of female mice heterozygous for X-linked coat colour genes.
        - The pigmentation of these heterozygous females was mottled, with large patches expressing the colour allele on one X and other patches expressing the allele on the other X.
            - This is typically the phenotypic pattern expected if different X chromosomes were inactive in adjacent patches of cells.



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    -
        - Similar mosaic patterns occur in the black and yellow-orange patches of female tortoiseshell and calico cats.
            - Such X-linked coat color patterns do not occur in male cats because all their cells contain the single maternal X chromosome and are therefore hemizygous for only one X-linked coat color allele.
    - Evidence* using clones of human fibroblast cells
        - Individual cells are isolated following biopsy and cultured in vitro.
        - The culture of cells derived from a single cell is called a clone
        - The synthesis of the enzyme glucose-6-phosphate dehydrogenase (G6PD) is controlled by an X-linked gene.

        - Numerous mutant alleles of this gene have been detected, and their gene products can be differentiated from the wt enzyme by their migration pattern in electrophoretic field.
    - Hypothesis predicts that if inactivation of an X-chromosome occurs randomly early in development, and thereafter all progeny cells have the same X chromosome inactivated as their progenitor, such a female should show two types of clones; each containing only one electrophoretic form of G6PD, in approximately equal proportions.
    - Mammalian females are mosaics for all heterozygous X-linked alleles-some areas of the body express only the maternally derived alleles, and others express only the paternally derived alleles.
    - *exp. Red-Green colorblindness.
        - An X-linked recessive disorder.
        - In humans, hemizygous males are fully color-blind in all retinal cells.
        - Heterozygous females display mosaic retinas, with patches of defective color perception.
        - Random inactivation of one or the other X chromosome early in the development of heterozygous females has led to these phenotypes.
- **The Mechanism of Inactivation**
    - Someone, either DNA, the attached histone proteins, or both, are chemically modified, silencing most genes that are part of that chromosome.
        - Once silenced, a memory is created that keeps the same homolog inactivated following chromosome replications and cell division.
        - This is known as imprinting: whereby expression of genes on one homolog, but not the other, is affected.
    - A region of the mammalian X chromosome is the major control unit for inactivation. This region is the X-inactivation centre, is located on the proximal end of the P arm in humans
        - Its genetic expression occurs only on the X chromosome that is inactivated.
        - It contains several putative regulatory units and four genes.
        - X-inactivation specific transcript (XIST) is now known to be a critical gene for X-inactivation.
    - Observations regarding the RNA that is transcribed from the XIST gene
        - The RNA product is quite large and does not encode a protein, thus, is not translated.
            - The RNA products of XIST spread over and coat the X chromosome bearing the gene that produced them.
            - Two other noncoding genes at the XIC locus, Tsix (an antisense partner of Xist) and Xite, are also believed to play important roles in X-inactivation
        - Transcription of Xist initially occurs at low levels on all X chromosomes.

            - As the inactivation process begins, hwever, transcription continues, and is enhanced, only on the X chromosome that becomes inactivated.
            - Transcription of XIST is the critical event in chromosome inactivation
    - **What causes all but one X chromosome to be inactivated?**
        - Maternal and paternal X chromosomes must first pair briefly and align at their XIC loci as a mechanism for counting the number of X chromosomes prior to X-inactivation.
        - Deletion of the Tsix gene contained in the Xic locus blocked X-X pairing and resulted in chaotic inactivation of 0,1,2 X chromosomes
- **Transgenes:** are artificially introduced into the organism.
    - The inactive X must associate with regions at the periphery of the nucleus to maintain a state of silenced gene expression.
        - In a majority of human female somatic cells the inactivated X, present as a Barr body, is observed attached to the nuclear envelope.

7.6| The Ratio of X Chromosomes to Sets of Autosomes Determines Sex in Drosophila

*males and females in drosophila have the same general sex-chromosome composition as humans (XY, XX), we might assume that the Y chromosome also causes maleness in these flies? True? NOPEEEEE

- The Y chromosome is not involved in sex determination in the drosophila organism
- The X chromosomes and autosomes together play a critical role in sex determination.
- Nondisjunction is the failure of paired chromosomes to segregate or separate during the anaphase stage of the first or second meiotic division.
    - The result is the production of two types of abnormal gametes, one of which contains an extra chromosome (n + 1)
    - The other of which lacks a chromosome (n-1)
    - Fertilization of such aberrant gametes with a haploid gamete produces 2n + 1 or 2n - 1 zygotes.
    - In humans, if nondisjunction involves the X chromosome, in addition to the normal complement of autosomes, both an XXY and an X0 sex-chromosome composition may result.
    - (the "0" signifies that neither a second X nor a Y chromosome is present, as occurs in X0 genotypes of individuals with Turner syndrome)
- The Y chromosome in Drosophila lacks male-determining factors, but since the X0 males were sterile, it does contain genetic information essential to male fertility.
    - The Y chromosome in Drosophila contains only about 20 protein-coding genes but mutations of these genes has significant impacts on regulating expression of hundreds of genes on other chromosomes, including genes on the X chromosome.

- Drosophila has a haploid number of 4 progeny, thereby possessing three pairs of autosomes in addition to its pair of sex chromosomes.
    - Triploid females apparently originate from rare diploid eggs fertilized by normal haploid sperm.
    - Triploid females have heavy-set bodies, coarse bristles, and coarse eyes, and they may be fertile.
        - Because of the odd number of each chromosome (3) during meiosis, a variety of different chromosome complements are distributed into gametes that give rise to offspring with a variety of abnormal chromosome composition.
- The critical factor in determining sex is the **RATIO of X chromosomes to the number of haploid sets of autosomes (A) present**.
    - Normal (2X:2A) and triploid (3X:3A) females each have a ratio equal to 1.0, and both are fertile.
    - As the ratio exceeds unity (3X:2A), or 1.5, 4 exp., what was once called a superfemale is produced.
    - **Metafemales:** because they are most often inviable.
- Normal (XY:2A) and sterile (X0:2A) males each have a ratio of 1:2, or 0.5.
    - When the ratio decreases to 1:3, or 0.33, as in the case of the XY:3A male, infertile **metamales** results.
    - Males between (0.5-1.0: X:A) are generally larger, and they exhibited a variety of morphological abnormalities and rudimentary bisexual gonads and genitalia.
    - They were invariably sterile and expressed both male and female morphology, thus being INTERSEXES.
- In Drosophila, factors that cause a fly to develop into a male are not located on the sex chromosomes but are instead found on the autosomes.
- Some female-determining factors, hwever, are located on the X chromosomes.
    - With respect to primary sex determination, male gametes containing one of each autosome plus a Y chromosome result in male offspring
    - This is not because of the presence of Y but because they fail to contribute an X chromosome.
- Genic balance theory: a threshold for maleness is reached when the X:A ratio is 1:2 (X:2A), but the presence of an additional X (XX:2A) alters the balance and results in female differentiation.
- Mutant genes in Drosophila involving sex-determination:
    - Transformer (tra); a single autosome gene could have a profound impact on sex determination.
    - Female homozygous for tra are transformed into sterile males, but homozygous males are unaffected.
    - Sex-lethal (Sxl); plays a critical role, serving as a “master switch” in sex determination.

            - Activation on the X-linked Sxl gene, relies on a ratio of X chromosomes to sets of autosomes that equals 1.0, is essential to female development.
            - In the absence of activation, made development occurs.
        - Depending on how the initial RNA transcript of dsx is processed (spliced) the resultant dsx protein activates either male or female specific genes required for sexual differentiation.
        - Each step in this regulatory cascade requires a form of processing called RNA splicing, in which portions of the RNA are removed and the remaining fragments are "spliced" back together prior to translation into a protein.
    - Alternative splicing; the RNA transcript may be spliced in different ways
    - A different RNA transcript is produced in females than in males.
        - In potential females, the transcript encodes a functional protein and initiates a cascade of regulatory gene expression, leading to female differentiation.
        - In potential males, the transcript encodes a nonfunctional protein, leading to a different pattern of gene activity, whereby male differentiation occurs.
- Dosage Compensation in Drosophila
    - Drosophila females contain two copies of X-linked genes whereas males contain only one copy, a dosage problem exists.
        - Male X-linked genes are transcribed twice the level of the comparable genes in females
        - If groups of X-linked genes are moved (translocated) to autosomes, dosage compensation still affects them, even though they are no longer part of the X chromosome.
        - @ least four autosomal genes found in mammals are known to be invoeld, under the same master-switch gene, Sxl, that induces female differentiation during sex determination.
            - Mutations in any of these genes severely reduce the increased expression of X-linked genes in males, causing lethality.
    - In males: the mechanism of increased genetic activity:
        - One of the autosomal genes, mle (maleless), encodes a protein that binds to numerous sites along the X chromosome, causing enhancement of genetic expression.
        - The products of three other autosomal genes also participate in and are required for mle binding.
        - Proteins called male-specific lethals (MSLs) have been shown to bind to gene-rich regions of the X to increase gene expression in male flies:
        - Dosage compensation complex (DCC): cluster of gene-activating protein(S) → a collective
    - The master-switch Sxl gene plays an important role during dosage compensation.

        - In XY flies, Sxl is inactive, therefore, the autosomal genes are activated, causing enhanced X chromosome activity.
        - On the other hand, Sxl is active in XX females and functions to inactivate one or more of the male-specific autosomal genes, perhaps mle.
        - By dampening the activity of these autosomal genes, it ensures that hey will not serve to double the expression of X-linked genes in females, which would further compound the dosage problem.
    - In Drosophila, a delicate balance of gene products is necessary to maintain normal development of both males and females.
- **Drosophila Mosaics**
    - **Bilateral gynandromorph;** one-half of its body (the left of the fly) has developed as a male and the other half (the right half) as a female.
    - How do we account for this intersex?
        - If a female zygote (heterozygous for white eye and miniature wing) were to lose one of its X chromosomes during the first mitotic division, the two cells would be of the XX and X0 constitution, respectively.
        - So, one cell would be female and the other would be male
        - Each of these cells is responsible for producing all progeny cells that make up either the right half or the left half of the body during embryogenesis
    - In the case of bilateral gynandromorph, the original cell of X0 constitution apparently produced only identical progeny cells and gave rise to the left half of the left, which is male (because of its chromosomal constitution)
        - Since the male half demonstrated the white miniature phenotype, the X chromosome bearing the w+, m+ alleles was lost, while the w, m-bearing homology was retained.
        - All cells on the right side of the body were derived from the original XX cell, leading to female development.
        - These cells, remaining heterozygous for both mutant genes, expressed the wt eye-wing phenotype.

7.7| Temperature Variation Controls Sex Determination in Reptiles

- Temperature-dependent sex determination (TSD)
- In many species of reptiles, GSD is involved at conception based on sex-chromosome composition.
- In boas and pythons, it is however impossible to distinguish one sex chromosome from the other in either sex.
- In other reptilian species, TSD is the norm, hwever, so crocodiles, most turtles, and some lizards - where sex-determination is achieved according to the incubation temperature of eggs during a critical period of embryonic development.
    - Case I: low temperatures = 100% females, high temperatures = 100% maleness.
    - Case II: low temps = 100% male, high temps = 100% females

    - Case II: low and high temperatures = various proportions of males.
        - This is seen in various species of crocodiles, turtles, and lizards.
    - Pivotal temperature: a temp at which approximately equal numbers of males and females results.
- What are the metabolic or physiological parameters affected by temperature that lead to the differentiation of one sex or the other?
    - Involves steroids (mainly estrogens) and enzymes involved in their synthesis.
    - The effects of temperature on estrogens, androgens, and inhibitors of the enzymes controlling their synthesis are involved in the sexual differentiation of ovaries and testes.
    - Aromatase (an enzyme) converts androgens (male h. Like testosterone) to estrogens (f.h. Like estradiol)
    - The activation of the enzyme is correlated with the pathway of reactions that occurs during gonadal differentiation activity and is high in developing ovaries and low in developing testes.
    - A thermosensitive factor mediates the transcription of the reptilian aromatase gene, leading to temperature-dependent sex determination.
    - Sex-determining mechanisms involving estrogens seem to be characteristic of nonmammalian vertebrates.
        - The regulation of these systems is controlled by sex chromosomes in many of these organisms.

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1. Sexual reproduction depends on the differentiation of male and female structures responsible for the production of male and female gametes, which in turn is controlled by specific genes, most often housed on specific sex-chromosomes.