Lec 1

- Cytogenics = Study of chromosomes
- double stranded DNA wrapped around histones = nucleosome
- packed nucleosomes = chromatin
- Chromatin embeded in scaffold = chromatid
- 2 sister chromatids = chromosome

Cell Cycle

- Interphase = G1, S, & G2
  - DNA is loose & accessable for proteins to be able to bind
- Before S phase, DNA is one double stranded chromatid (still called chromasome)
- G phase is growth phase, where RNA polymerase makes more mRNA & proteins nessesery for S phase
- Sphase is "Synthesis" phase, where DNA polymerase replicates double stranded Chromatid to make a sister chromatid joined at the centromere

* Chromosome enters S phase as one chromatid & come out as 2 chromatids

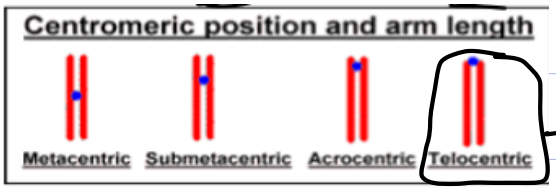
- M phase is mitosis, DNA is tightly packed for easier separation of chromosomes

* most condensed DNA in metaphase

- chromosome = condensed chromatin

Nomenclature of Chromosomes

- based on centromere position & arm length
- upper arm = P (shorter) ; lower arm = q (longer)



→ abnormal no p arm

Human Chromosome

- 23 pairs of chromosomes, from mom & Dad
- 22 pairs of diploid (Somatic cells), 1 pair sex chromosome XX or XY (haploid)

Mitosis

- division of cells to produce identical daughter cells in terms of DNA sequence, not cytoplasm
- 5 phases:

Prophase

chromosomes begin to condense, nuclear envelope collapse, centrioles appear on opposite poles

Metaphase

chromosomes individually allign on metaphase plate

Anaphase

Separation of sister chromatids

Telophase

nuclear envelope forms, 2 daughter nuclei form, chromosomes diffuse & cleavage furrow forms

Cytokinesis

division of cytoplasm

Meiosis

- produces daughter cells w/ ½ # of chromosomes & different DNA sequences (only egg & sperm)
- 2 stages : meiosis 1 = separation of homologous chromosome
  meiosis 2 = separation of sister chromatids

Meiosis 1

Prophase 1

condensation, envelope disapears, centrioles,
Recombination

* takes most time of all phases

Metaphase 1

homologous pairs line up

Anaphase 1

disjunction of homologous pairs

* chromosome # reduced by ½

Telophase 1

two haploid cells separate

Meiosis 2

- immediatly after meiosis 1 (no interphase)
- same steps

Anaphase 2

separation of sister chromatids

- final product is 4 non identical daughter cells (egg/sperm)

Prophase 1

- Recombination occurs
  - exchange of genetic material between non sister chromatids of homologous chromosomes
- sub divided to 5 stages

Leptotene

initiation of condensation, homologous chromosomes align for recombination

Zygotene

Synapsis & tetrad formation

Pachytene

Synapsis is complete, each homologous pair is called a tetrad, Recombination occurs

Diplotene

Chromosomes separate but stay bound at chiasmata

Diakinesis

further condensation until it reaches metaphase

Gregor Mendel laws

- law of segregation: homologous chromosomes separate & appear in different daughter cells
- law of independent assortment: chromosomes align on metaphase plate independently so traits are inherited independently of each other

* most of cell cycle taken by Interphase

Lec 2

- each chromosome has genes which are segments of DNA that carry info for a specific trait; & different versions of genes are called alleles
- possible genetic combinations are calculated by 2^n
  n = # of chromosome pairs (2^{23})
- meiosis gives genetic diversity b/c of independent assortment & genetic Recombination in meiosis 1

Why Study Chromosomes?

- Clinical conditions are linked to chromosome abnormalities
- chromosome abnormalities are seen in:
  - 20-27% of sex reversal / puberty abnormalities
  - 33-67% of spontaneous miscarage
  - 2-5% multiple miscarage
  - majority of leukemia & solid tumors

How to Study Chromosomes

- extraction of chromosome depends on disease & purpose of study
- Amniotic fluid cells used for embryonic genes
- Bone marrow for diseases in blood
- Peripheral blood for lymphocytes
- Skin or organ biopsy
- A Karyotye is a diagram of ordered arrangement of chromosomes by hight (1 being longest & 22 being the shortest, followed by sex chromosomes)

- images are obtained during metaphase in a dividing cell
- An idiogram is a diagrammatic representation of the karyotype to show size & banding pattern of chromosomes
- each chromosome has different banding patterns
- banding pattern of same chromosome is different depending on banding method

So what do we do after obtaining a sample ?

- because cells are usually in interphase (loose diffuse chromatin) we need to induce the cell cycle, & stop it in metaphase .... how ?

1) sample obtained is placed in media to keep sample alive, & a mitogen (phytohemoglutanin) is added to induce cell cycle
2) then a mitotic inhibitor (colchicine) is added to stop the cell in metaphase
   * colchicine prevents assembly & polymerization of microtubule fillaments of centrosome
3) centrifuge @ low speed to remove media
4) hypotonic solution added to swell cells
5) cells placed on slides burst & chromosomes scatter
6) Giemsa stain is added

Notes before next section:

- Adenine & Thymine have 2 H-bonds (A&T)
- Guanine & Cytosine have 3 H-bonds (G&C)
- gene rich region = euchromatic (mainly G & C)
- gene poor region = heterochromatic (mainly A & T)

this is because AT areas have tissue specific genes while GC areas have housekeeping genes

- trypsin is a digestive enzyme that degrades peptide bonds & digest chromasomal proteins to relax chromatin structure so stain can access DNA

G-Banding Technique

- chromosomes treated w/ trypsin first
- Giemsa stain is added

* Giemsa forms hydrophobic interractions @ hydrogen binding site, & favors AT regions more
- Giemsa binds AT stronger than GC, so AT regions show darker (black) stain
- produces 300-400 bands & each band has seveal-10 million nucleotides

R-Banding Technique

- the "Reverse" of G banding, so euchromatic areas are stained dark (GC) ... why?
- b/c first we heat the sample, causing degridation of AT hydrogen bonds so Giemsa stains GC regions darker

- flourochrome is added to sharpen or enhance color
  - used in G-banding to identify deletion mutations & in R-banding to confirm findings of G-banding

Chromosome Shape

- Metacentric, Submetacentric, Acrocentric
- q & p arm are numbered starting from centromere ending @ telomere

Acrocentric

- chromosomes 13-15, 21 & 22
- p-arm has 2 regions
  1) Satellite: heterochromatic region, darkly stained, repetative sequence of base pairs
  2) Stalk: euchromatic, lightly stained, codes for rRNA
- p-arm has no clinical significance so damage to it wont affect anything... other acrocentric chromosomes can compensate & make rRNA

High Resolution Banding

- damage to about 300 million nucleotides can be detected by G & R banding, but damage to less, can not

what do we do?

- obtain sample from less condensed chromatid (prophase / pro-metaphase)
- # of banding increases from 300-450 or even 800
- So we now can detect less obvious abnormalities

Chromosome Structure

Centromeres

- Structural heterochromatic DNA required for chromosome segregation
- tandem repeats of 171 base pairs called alpha satelite
- all chromosomes have same alpha satelite

Telomeres

- at ends of eukaryotic chromosomes
- TTAGGG repeats thousands of time
- structural region that prevents end to end fusion of chromsomes
- Replicated by DNA polymerase & telomerase
- as you age, telomerase activity ↓
- telomeres get shorter each time cells divide until its gone & cell is innactive
- protects us from cancer

Sub-telomeric Region

- repeats ase not universal like telomeres
- some homology, but not identical among chromosomes

Lec 3

Aneuploidy

- Chromosome number thats not an exact multiple of haploid number (2n)

- trisomy : extra chromosome
- monosomy : absence of a chromosome
- change in chromosome * due to nondisjunction
- if nondisjunction happens in meiosis 1 then homologous chromosomes dont separate
  - ½ the gametes have extra chromosome (n+1) & other ½ is missing a chromosome (n-1)
- if in meiosis 2, then sister chromatids dont separate
  - ½ carry correct * (n), ¼ has extra (n+1), ¼ is missing 1 (n-1)

* nondisjunction of mitosis is the same as meiosis 2

Polyploidy / Euploidy

- more than 2 complete sets of chromosomes
  - extra, but exact copy of n
- Triploidy (3n)
- tetraploidy (4n)
- polyploidy more common in plants
- polyploids are more normal in appearance than aneuploids

Alteration of Chromosome Structure

- Deletion: remove chromosome segment
- Duplication: repeats segment
- inversion: reversing orientation of segment on same chromosome
- Translocation: moving segment from one chromosome to another

- Alteration of chromosomes leads to serious disorders
- Some aneuploidy disrupts genetic balance more than others, so some people can survive but suffer from syndromes

Down Syndrome (Trisomy 21)

- 3 copies of chromosome 21
- 1/700 children
- correlated to pregnancy at advanced age

Clinically

- mental retardation
- low nasal bridge
- hypotonia
- up slanting palpebral fissures
- low set ears
- congenital ♡ disease (VSD, AVD)
- eyefolds, protruding tongue, intestinal problems, 15 fold ↑ risk of leukemia
- Simian line

* features easily recognized at Birth

- non disjunction can occur in meiosis 1 or 2
- 94% due to maternal error
  - 64% in meiosis 1, 19% in meiosis 2, 11% intermediate
- 4.5% due to paternal error
  - 1% meiosis 1, 3.5% meiosis 2, 1.5% unknown

how do we determine if the extra chromosome came from mother or father?

- we look at repetative non coding regions of DNA
- these regions differ from person to person, so it is used as a polymorphic marker

Partial Trisomy 21 (21q)

- 2 fused q arms at chromosome 21, instead of 1 q arm (46, XX, 21 q+)
- phenotypically down syndrome

Edward Syndrome (Trisomy 18)

- CHD
- failure to thrive
- retarded (mental & growth)
- hypertonia
- pominant occiput
- low set ears
- intestine problems
- clenched fist
- rockerbottom feet

Patau Syndrome (trisomy 13)

- CHD
- scalp defects
- retarded
- hyper / hypo tonia
- microcephaly

- low set ears
- Small eyes
- Cleft lip / palate
- rocker bottom feet
- poly / syndactyly
- polycystic kidneys