Lecture 12
10/1/12

In G2, DNA 2x, then M where it divides and goes back to X

Mitosis

1. Interphase: chromosomes are relaxed. (G1-G2)
   Microtubules that form spindles; move the chromosomes. Pull sister chromatids apart and partition them.
2. Metaphase: chromosomes have condensed and formed sister chromatids.
   Chromosome being pulled by microtubules to other sides while lined in the middle.

Visualization of Chromosomes:

Interested in chromosome patterns

1) Begin with growing cells to observe cell in mitosis.
Must be rapidly growing.
Only small fraction of the cells will actually be in mitosis. Must maximize cells in mitosis to observe chromosomes. Beginning of mitosis, chromosomes condense. Fibers/microtubules begin to pull chromosomes.

2) Colchicine (plant extract) 12 -20 hours. Will disrupt microtubules to fall apart into their subunits. G1 and G2 will continue onto replicating and into mitosis. If in Mitosis, cells will freeze in mitosis because spindle fibers cannot move. Population of cells in Mitosis will increase in number, increasing the chance of seeing chromosomes in mitosis.

3) Must break apart suspension of cells. Mash cells over slide. Wash with methanol-acetic acid. Removes lipids and makes DNA accessible.

4) Use trypsin solution (continuing protease) digests some o the proteins holding chromosomes together.

5) Giemsa stain.

**same enzyme phosphorylating lamina will also cause the chromosome to condense.
Activates enzyme that causes centromeres to split (chromsomes to split) by phosphorylation= **MPF**

gather growing cells → incubated with colchicine → cells in mitosis (prophase) accumulate → use methanol-acetic to remove lipids → Trypsin to digest proteins/centromere → Giemsa stain

Special Karyotyping: SKY
Look for unique sequences t o chromosome 1. Synthesize segments/stretches DNA complementary to the chromosome using a DNA sysnthesizer. **Oligonucleotides** (~35 base pairs)

Now have accumulated 24 pairs of these sequences from 24 chromsomes (including Y).
On chromosome 1, link dye that fluoresces yellow. Dye ataches covalently. Chromosome 5, all oligonuclueotides will fluoresce yellow-orange. Chromosome 8, will fluoresce light yellow.

Must denature DNA to help separate and relax double helix.

Translocation: piece of chromosome that migrates to another chromosome

Chronic Myeloid Leukemia: progressive. First shows itself as increase in number of leukocytes (granula)
Over period of 5 years (chronic phase) leukocytes continue to increase without any intervention. Organs begin to become compromised (kidney failure or respiratory infection)

Decrease in density of bone cells, and increased in number.

How to recognize disease early on? Karyotype analysis. Bone marrow biopsy.

Pattern:

- Shortened chromosome 22, reciprocal translocation between 9 and 22
- **Nowell & Hungerford** (1960) – called Philadelphia chromosome

Mosaic Gene: BCR-ABL: codes for protein tthat is a mosaic. Chrom amino end to carbonyl end: BCR – ABL protiens
Proteins unable to be regulated, always on.
Some drugs selectivey inhibit CDK cyclin
Gluvec

BCR from chromosome 22, ABL from chrom 9

Lecture 13
10/3/12

Proteins can cross in a selective manner, specified by proteins embedded in membrane
Not always energy requiring process, can be used to regulate physiological functions

Movements across membrane:

Diffusion:
At equilibrium, concentrations inside and outside the vesicle/cell match

Simple diffusion: no carrier, channel. Nothing intervening between substance and membrane

Carrier-mediated diffusion: some sort of protein embedded in membrane speeds up process of diffusion

What determines rate of entry and exit? Rate of collision to membrane
V(out to in) = proportional to concentration outside of cell
V flux ~ – (proportional to) =number of particles/area (micrometers squared)*seconds
S0 = outside
S'0 = inside

Net flux V = V(out to in) – V'(in to out) = K([S0] – [S'0])

k= diffusion coefficient. Find rate of exit and entry to be linear

<u>Measuring Rate of Entry</u>: Using whole cells

1. Take sample of RBCs that have been isolated in solution containing glucose.
2. RBCs require glucose for cell processes.
3. Allow to incubate for as little as possible. <1 minute.
4. Collect cells and measure glucose concentration inside RBC compared to initial concentration

[some cells already have glucose in cells. How do you measure glucose in cells?]

- Use radioactive form of glucose
- Or generate **<u>Ghosts</u>**. Place RBCs in water. RBC's will swell up and enzymes and internal structures will leak out. Place back in isotonic solution to regain normal size. RBC now is empty except with transport membranes.
- Or: generate artificial membranes.

Simple vs Facilitated Diffusion:

- Simple, linear line.
- Facilitated; rate maximizes and will level off . Becomes saturated.

One of the clear indications of facilitated diffusion is that you will get saturation.
Channel: glycerol uptake in E. coli
Has aqueous channel to allow movement of specific molecules.

- Glycine
- E.coli/ bacteria: plasma membrane, cell wall
- Glycerol – moves with mechanism involving a carrier. Found to be protein embedded in plasma membrane → glycerol channel. Found to be **<u>aquaporin</u>**, "water channel"
- **<u>Periplasm</u>**: space between cytoplasm and plasma membrane
- Channel consists of alpha helix spanning membrane, loop with alpha helix. **6 helices that span membrane and 2 loops. 4 subunits to make 4 channels. Loops inside channel determine specificity of channel.**
- **6 alpha helices give wall of cylinder. 2 loops (one from cytoplasm end, one from extracellular end) inside channel**
- presence of internal loops provide specificity.
- Serves as transport of water in kidneys and urine excretion.