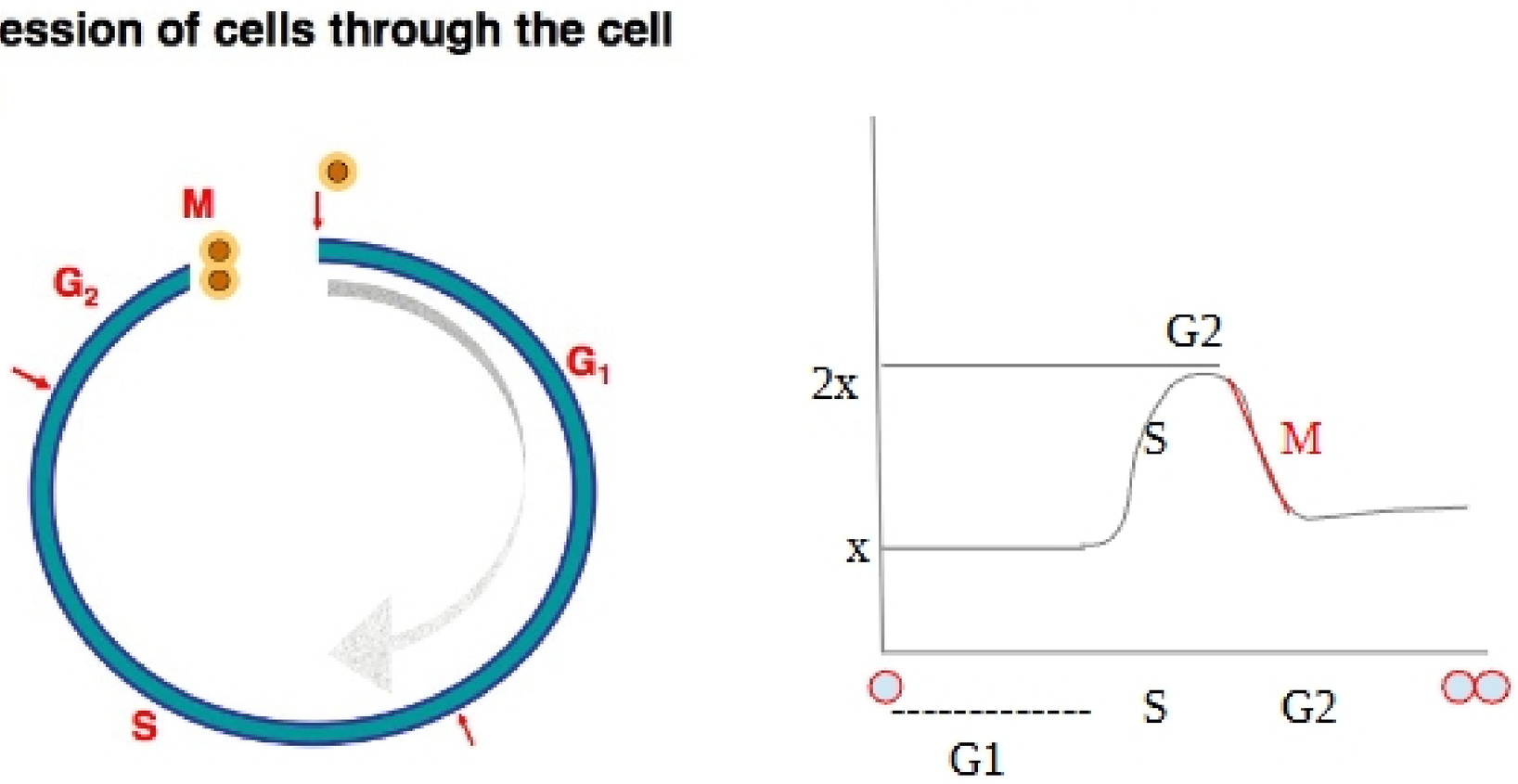
BSCI330 Exam 2 notes

Lecture 10/1

Karyotype Analysis
- cells in mitosis become visible to count chromosomes #, abnormality, breakage
- helps identify malignancy, condensed form

How to Detect Chromosome #
Stages of Eukaryotic Cell (after mitosis before split into two daughter cells)



cells rapidly proliferate

- G1 = begin proliferations proliferation, begin replication of DNA
- S = doubles DNA content
- G2 = splits chromosome → 2 daughter cells
- liver cells will proliferate if damaged
- present in G1 (waiting for stimulus to start process)
- karyotype viewed in M phase
- stem cell research taken from infants because cells ready to proliferate in G1 phase

Phases of Mitosis
- microtubules = fibers that can pull two sister chromatids apart
- polymers of microtubules fall apart and become fibers to split sister chromatids (metaphase)
- form spindles and serve as tracts for vesicles
- during mitosis fibers in cells breakdown to form after division

Visualization of Chromosomes
growing population of cells = (only small fraction in mitosis, procedure wants to maximize chromosomes in mitosis)
⇩
colchicine 12-20 hrs = (added to solution after cells have been rapidly growing, disrupts microtubules no matter what stage the cell is in, inhibits proliferation and cell's don't die)
⇩
accumulate cells in = all phases will continue except cells in M phase will be frozen
mitosis (prophase)
⇩

fix cells in methanol- = drop suspension of cells on to slide, smash on cover slip and add MAA which
acetic acid eliminates some lipids and makes DNA more available

⇩

trypsin solution 10-15 min = protease, digests proteins that held DNA together

⇩

stain with Giemsa = stain

(colchicine used in cancer treament because stops cells from proliferation)

When looking under microscope

- many cells with many nuclei on slide
- chromosomes spread on surface
- cut chromosomes
- look at lengths, staining, #, to detect which chromosome

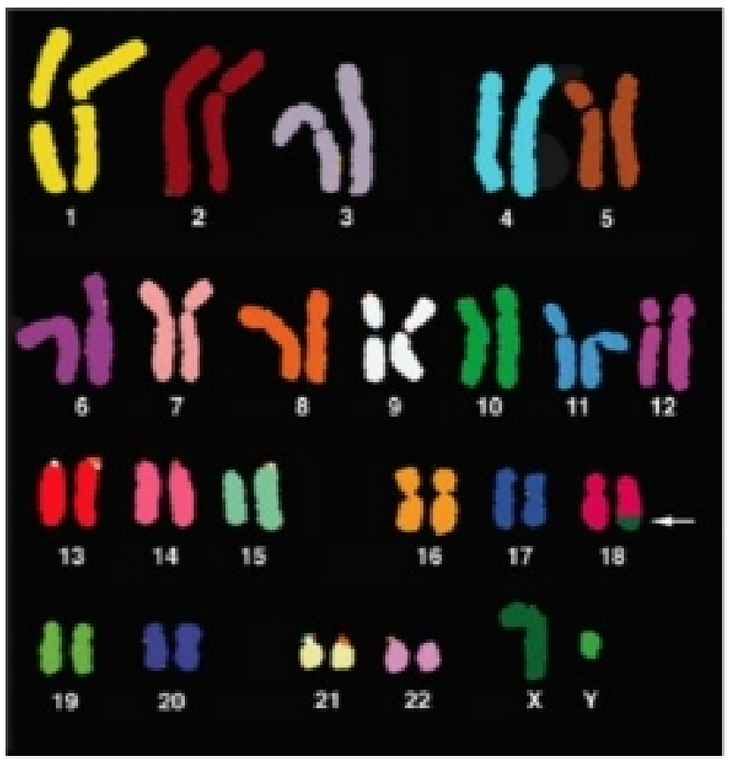
MPF – Mitosis promoting factor

- cyclin β – CDK1
- staining pattern unique for chromosomes

Spectral Karyotyping

- double stranded DNA
- denature DNA so relaxes condensation
- synthesize segments complementary to those stretches
- linker dye so all oligonucleotides will fluoresce identifying chromosome uniquely
- incubate cells with mixture of oligonucleotides and dip chromosomes in
- complementary pairs will attach
- sensitive to translocations

Chromosome 18 – translocation = piece of chromosome broke off and lygated to other chromosome



Chronic Myeloid Leukemia (Nowell and Hungerford)

- density of cells much smaller
- lethal and progressive disorder
- increase in number of leukocytes (not alarming unless persists for a few months)
- increase for 5 years without intervention then WBC are so high and organs are destroyed (secondary disoder)
- ex. kidney failure because too many cells and proteins are released taking up too much space for other cells
- early detection through Bone Marrow biopsy
  - cells taken → caused to proliferate → view under microscope → if chromosome 22 is shortened and reciprocal translocation with 9 and 22 = indicates CML

Mosaic gene #22: BCR ● ABL: #9

- when BCR and ABL come together gene is always on
- amino (NH3) | BCR|ABL| (COO-) carboxylic
- kinase indicates cells to proliferate

SOLUTION

- Gluvec blocks ABL (inhibits kinase/cell proliferation)

- and bone marrow transplant

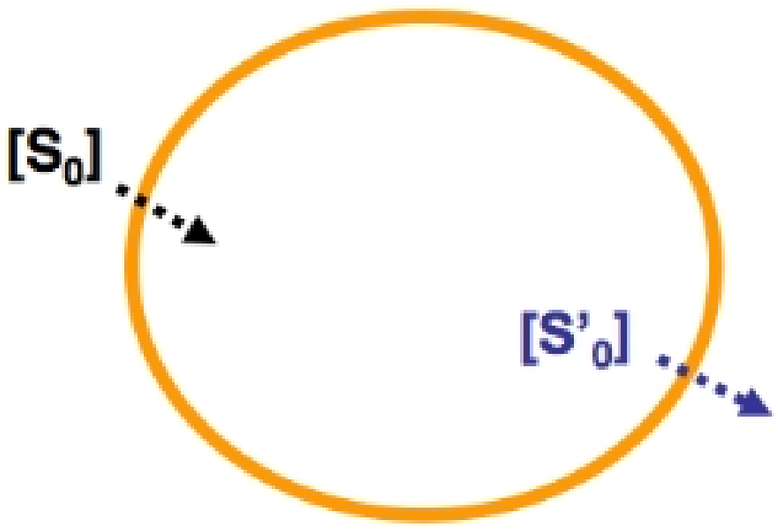
Lecture 10/3

Movements Across Membrane

1) Diffusion
   - moves toward equilibrium
   - not energy costing
   - redistribution of molecules across membrane
   - simple
     - equilibrium reached with time
     - concentration of molecule inside and out are equal
   - carrier-mediated
     - protein embedded in membrane that speeds up process

How to tell:

$V_{\underline{out\ to\ in}}$ is proportional to $[S_0]$



$V'_{\underline{in\ to\ out}}$ is proportional to $[S'_0]$

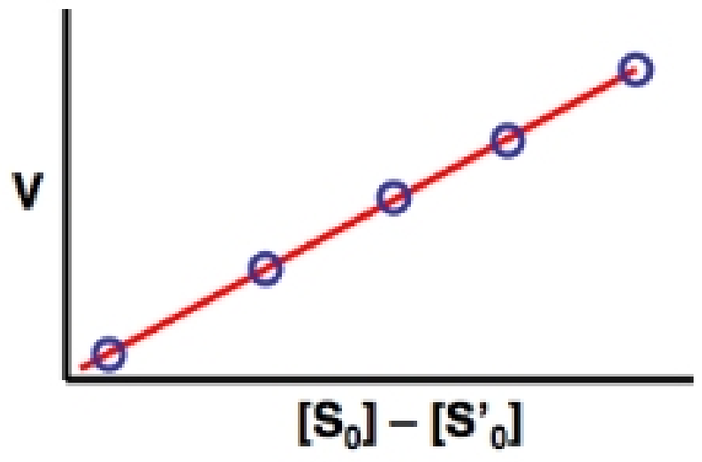
Passive Diffusion:

- flux = # of particles moving across certain area over time
- constant related to shape of molecule/temp
- rate is linear
- in order for substances to move, molecules must bump each other into the membrane
- rate of bump movement proportional to concentration
- area in μ^2 x sec

$V_{\underline{out\ to\ in}}$ is proportional to $[S_0]$

$V'_{\underline{in\ to\ out}}$ is proportional to $[S'_0]$

Net flux $V = V_{\underline{out\ to\ in}} - V'_{\underline{in\ to\ out}} = K([S_0] - [S'_0])$



How to Measure the Rate of Entry

- RBC in solution with glucose
- want to generate linear model based on concentration difference across cell-surface
- saturation kinetics