Lecture #1

1. Calculate the number of generations to extinction of a gene for a native protein in mice that does not confer protection to a lethal disease in the presence of a mutant that will protect against the disease. Assume that 90% of the homozygous native protein animals die, whereas 60% of the heterozygous animals and 10% of the homozygous mutant animals die if an epidemic of the disease strikes. When the epidemic first strikes, the population is 10,000 with 20% of the mutant gene. Because the natural resources are not limiting, the animals that survive the disease will double in number each generation. The disease strikes once in each generation on average. (Remember that you can't have fractions of animals and if the calculations say that 7.9 animals will survive, then in reality only 7 do survive. Less that 1 means 0 or extinction). To give a hint, there are 400 homozygous mutant, 3200 heterozygous, and 6400 homozygous native animals before the first epidemic; and after the first epidemic, the number drops to 360 homozygous mutant, 1280 heterozygous, and 640 homozygous native animals.

Assuming Mendel genetics during replication we have doubling of the gene pools, not doubling of each of heterozygosis and homozygosis subpopulations independently.
P_i – total size of population at the generation # i
M_i – number of mutant animals at the generation # i
H_i – number of heterozygote animals at the generation # i
N_i – number of native animals at the generation # i

m_i – frequency of mutant gene in population at the generation # i
n_i – frequency of native gene in population at the generation # i

$m_i+n_i=1$, for any i
$(m_i+n_i)^2=1$
$m_i^2+2*m_i*n_i+n_i^2=1$

m_i^2 – frequency of mutant homozygote animals in population
$2*m_i*n_i$ – frequency of heterozygote animals in population
n_i^2 – frequency of native homozygote animals in population

At the beginning (i=0): m=0.2, n=0.8

At the each step we can calculate size of each population by knowing the frequency of genes:
$M_i = m_i^2*P_i$
$H_i = 2*m_i*n_i*P_i$
$N_i = n_i^2*P_i$

Epidemic strikes and we need to recalculate size subpopulations according to survival probability for each subgroup ([survival probability] = 1-[probability to die]):

M_i(after epidemic)=0.1*M_i
H_i(after epidemic)=0.4*H_i
N_i(after epidemic)=0.9*N_i

Then we can recalculate frequencies of different genotypes:
$M_i/P_i = m_i^2$
$H_i/P_i = 2*m_i*n_i$
$N_i/P_i = n_i^2$

Then population doubles with each generation:
$P_{i+1}=2*P_i$

By repeating this calculations for each generation, we can find that native homozygote animals disappear ($N_i<1$) in ~6 generation, and heterozygous in ~27 generations ($H_i<1$).

Lecture #2

1. In a recent article by Dayel et al (1999) they measure the diffusion of a green fluorescent protein by fluorescence recovery after photobleaching in the lumen of the endoplasmic reticulum (ER) and in cytoplasm and find a 3 fold higher diffusion coefficient in cytoplasm (1.5×10^{-7} cm2/sec) than in the ER. My lab has measured diffusion of small (0.2 micron diameter) beads along microtubules (Wang and Sheetz, 2000) using single particle tracking and have found a one-dimensional diffusion constant of 2×10^{-10} cm^2/sec. Two proteins (both 5 nm in diameter) are synthesized in cytoplasm at a rate of 1000 molecules per minute in a thin region of cytoplasm (many lamellipodia are only 0.2 microns thick) that is essentially two dimensional. One protein, S, is soluble and the other, M, is rapidly bound to the microtubule. After 100 seconds of diffusion (assume that all molecules are at one point and start diffusing at t = 0), what is the average displacement of protein S and of protein M? Remembering that the distribution is aussian, what are the average concentrations of the two proteins within the region from he origin to the average displacement point? Now calculate the average displacement and concentration, if 1000 molecules of soluble protein, S, are expressed with a signal sequence so that they are translocated into the ER lumen which is a tube 0.1 microns in diameter and hundreds of microns in length. Answer: After 100 sec., $\Delta X = \pm 7.6 \times 10^{-3}$ cm for S and $\pm 2.0 \times 10^{-4}$ cm for M. The volume for S is a cylinder 37 microns in radius and 0.2 microns thick ($2\pi r2\ l = 7.2\ 10^{-9}$ cm^3) whereas for M the volume is a cylinder of the microtubule plus the protein ($2\pi r2\ l = 3.8\ 10^{-15}$ cm^3).

For Protein M, diffusion is one-dimensional, and is mathematically defined by: $2D*t = \langle \Delta X^2 \rangle$, where $D = 2 \times 10^{-10}$ cm^2/s and t = 100 s

$\langle ?X^2 \rangle = 2D_{1\text{-}D}t = 2 * 2 \times 10^{-10}$ $cm^2/s * 100$ s $= 4 \times 10^{-8}$ cm^2
$\langle ?X \rangle = 2 \times 10^{-4}$ cm

For Protein S, diffusion is two-dimensional, and is mathematically defined by:
$4D_{2\text{-}D}t = \langle ?X^2 + ?Y^2 \rangle$, where $D_{2\text{-}D} = 1.5 \times 10^{-7}$ cm^2/s, t = 100 s, and $\langle ?X^2 + ?Y^2 \rangle = \langle ?R^2 \rangle$, where R is the radius of diffusion.

$\langle ?R^2 \rangle = 4D_{2\text{-}D}t = 4 * 1.5 \times 10^{-7}$ $cm^2/s * 100$ s $= 6 \times 10^{-5}$ cm^2
$\langle ?R \rangle = 7.7 \times 10^{-3}$ cm

Assuming that all proteins at time t = 0 are at the origin, then protein diffuses radially and protein distribution is a Gaussian curve, and 68% of the total concentration is contained within the region to the average displacement point. If we take the total volume to be $p\langle ?X \rangle^2 * .2 \times 10^{-6}$ m (thickness of cell) and $p\langle ?R \rangle^2 * .2 \times 10^{-6}$ m:

Protein M:
Average Concentration = 1000 molecules/min * 1 min/60 s * 100 s * 1 mol/6.02×10^{23} molecules * 1/[$p(2.0 \times 10^{-4}$ cm * 1 m/100 cm$)^2 * .2 \times 10^{-6}$ m] * 1 m^3/1000 l * .68
Average Protein Concentration = 7.5×10^{-7} M

Protein S:

Average Concentration = 1000 molecules/min * 1 min/60 s * 100 s * 1 mol/6.02 x 10^{23} molecules * 1/[p(7.7 x 10^{-3} cm * 1 m/100 cm)^2 * .2 x 10^{-6} m] * 1 m^3/1000 l *0.68
Average Protein Concentration = 5.1 x 10^{-10} M

S-protein translocated into the ER lumen:
This is essentially a one-dimensional distribution (ER lumen – thin long tube), or $2D_1t = <?x^2> = 2*(5*10^{-8}cm^2/sec)*100sec$
$?x = 3.2*10^{-3}cm$

1000*68%=680 molecules
Volume for concentration calculation is the volume of one ER lumen with diameter 0.1um and length <Δx>:
$680/(N_a*\pi*(0.05um)^2*3.2*10^{-3}cm) = \sim 4.5*10^{-6}M$

2. A neuron (70 microns in diameter) is sprouting an axonal process and there are new proteins synthesized that are needed at the tip of the growing process. If a protein is synthesized (1000 molecules) at the cell end of an axon that is one hundred microns long (one micron in diameter) and the protein has a diffusion coefficient of 2 x 10^{-7} cm^2/sec, the average displacement of the protein molecules will equal the length of the axon after how many seconds? What will be the average protein concentration in the axon after that time? Now repeat the calculations for axons that are 1 mm and 1 m long (remember that you may have some axons a meter in length). If there is a flow of cytoplasm into the axon as it is growing (the axon is elongating at 1 micron per minute typically), what will be the root- mean square average displacement of the 1000 molecules of protein that are placed at the end of a 1mm axon after 100 minutes?

Diffusion is one-dimensional, because the proteins are directed along the length of the axon.
$2D_{1\text{-}D}t = <\Delta X^2>$, $D_{1\text{-}D}$ = 2 x 10^{-7} cm^2/sec and X = 100 x 10^{-6} m

$t = <\Delta X^2> / 2 D_{1\text{-}D}$
t= (100 x 10^{-6} m)^2 / 2(2 x 10^{-7} cm^2/sec * 1 $m^2/100^2$ cm^2)
t= 250 seconds

If we assume that the neuron is spherical, with a radius of 35 μm, then the volume = $^4/_3$ pr^3.- in case of short axon we can omit volume of axon:

Average Concentration = 1000 molecules * 1 mol/6.02 x 10^{23} molecules * 1/[$^4/_3$ p(35 x 10^{-6} m)^3] * 1 m^3/1000 l
Average Protein Concentration = ~ 10^{-11} M

For <?X> = 1 x 10^{-3} m
t= (1 x 10^{-3} m)^2 / 2(2 x 10^{-7} cm^2/sec * 1 $m^2/100^2$ cm^2)
t = 2.5 x 10^4 seconds

In this case the volume of cell body is still much bigger then volume of axon and the average concentration will be the same

For X = 1 m
$t = (1\ m)^2 / 2(2 \times 10^{-7}\ cm^2/sec * 1\ m^2/100^2\ cm^2)$
$t = 2.5 \times 10^{10}$ seconds

Here is the volume of axon is bigger then volume of cell body and protein will be redistributed into axon by Gaussian distribution:
Average Concentration = 1000molecules*1 mol/6.02×10^{23} molecules*1/[p*1m*$(0.5um)^2$]*0.68 = ~$1.43*10^{-12}$ M

If there is a flow of cytoplasm into the axon as it grows, then average displacement is:

t = 100 minutes and v = 1×10^{-6} m/minute

$\langle \Delta X^2 \rangle = 2D_{1\text{-}D}t + (vt)^2$
$\langle \Delta X^2 \rangle = 2 * 2 \times 10^{-7}\ cm^2/sec * 1\ m^2/100^2\ cm^2 * 100$ minutes * 60 s/1 minute
$+ (1 \times 10^{-6}$ m/minute * 100 minutes$)^2$
$\langle \Delta X^2 \rangle = 2.5 \times 10^{-7}\ m^2$
$\Delta X = 5*10^{-4}m=100um$

Lecture #3

1. If we assume that a protein ($D = 2 \times 10^{-7}$ cm^2/s) is synthesized at a steady state rate of 100 molecules per second and assembled into a larger complex at the same rate, then you can calculate the gradient of protein concentration in the cell from a site of synthesis separated by 20 microns from the site of assembly (assume that all the molecules are moving down a 20 micron diameter tube). If the concentration at the site of assembly is 10^{-9} M and the assembly site is at the pole of a spherical cell of 40 microns in diameter, what is the concentration at the synthesis site at the center of the cell and what is the concentration in the other half of the cell?

Answer:

There is 1D diffusion down 20μm-diameter tube with length L=20μm.

The flux is J=(dN/dt)/S, cross-section $S=\pi r^2$ (r=10μm, rate of synthesis dN/dt =(100 molecules/s)/N_a (normalization to Avagadro number) or 5.29×10^{-17} moles/cm^2/s

Gradient can be calculated by:

$J_x = -D\, dC/dx$ or $J_x/D = -\, dC/dx$

5.29×10^{-17} moles/cm^2/s /2×10^{-7} cm^2/s = 2.64×10^{-10} moles/cm^4 or 2.64×10^{-7} M/cm or 2.64×10^{-10} M/10μm

Changing of concentration is $\Delta C = L*dC/dx$, L – distance between sites of synthesis and assembly

Concentration at the center of cell: $C_{synthesis}=C_{assembly} +\Delta C$ Thus, the gradient is from 1.1 10^{-9} M to 1.0 10^{-9} M from site of synthesis to assembly

2. Many believe that the reduction in the number of dimensions of diffusion (e.g. from 3-D solution phase to a 2-D membrane phase or to a 1-D filament phase) will result in an increase in the rate of transport. How will problem 1 change if a microtubule extends from the site of synthesis to the site of assembly and all of the protein binds to the microtubule surface and diffuses with the same D as in Problem 1? Often the penalty for the binding of a protein to a filament or a membrane is a decrease in the diffusion coefficient. What would happen if the D is decreased by two orders of magnitude?

The cross-section of the flux-tube decreases and the flux dramatically increases (if the radius of microtubule is ~ 10nm, then increasing is proportional to the ratio of cross-sections: $S_{tube}/S_{microtubule}= 10^6$). This leads to an increased concentration gradient (also 10^6-fold higher) and the calculated concentration at the center of cell on the microtubule would be 2.64×10^{-4} M. However, in the cytoplasm, the concentration would be effectively zero. The decreasing of D by 2 orders of magnitude still leads to increasing of the concentration gradient (10^2-fold increase).

3. Axonal transport has been studied extensively by injecting the cell bodies with radioactive amino acids and then following the distribution of radioactivity in specific

proteins along the axon ((Jung et al., 2000; Nixon et al., 1994; Dillman et al., 1996)). Originally the slow axonal transport of cytoskeletal proteins (1-2 mm/day) was interpreted as the bulk movement of the assembled cytoskeleton down the axon. In the case of studies of the rat optic nerve (radioactive amino acids were injected into the eye and the optic nerves, 1.6 cm in length, were cut up at various times after injection), we can compare three different models of the transport process. 1) active transport; in this case assume that the cytoskeletal proteins are carried in a complex that moves at the rate of 2 mm/day and all of the radioactive amino acids (10,000 counts per minute (cpm) total) were incorporated into protein within one hour. 2) diffusive transport; assume that the protein has a diffusion coefficient of 10^{-7} cm^2/sec. 3) Rapid transport and diffusion model; assume that the protein is part of a complex which moves at a fast axonal transport rate (200 mm/day) but for only 1% of the time. For the rest of the time the particles diffuse at a rate of 10^{-10} cm^2/sec. Please calculate the number of cpms that would be expected in 2 mm slices of the axon for each of these cases after 1.5 days and one week.

Answer:
The cpm can be considered as measures of the amount of protein and we can use cpms as the concentration in diffusion calculations. We presume that time constant of radioactive decay is much slower than time of experiment.

1) Active transport v=2mm/day, so in 1.5 days all labeled aminoacids will be transported in 3 mm (all 10000 cpms in second 2mm slice). In 7 days all cpms will be in 7^{th} slice.
2) In case of diffusion there is one-dimensional 1D diffusion of radio-labeled compound from the cell body down to axon. We have Gaussian distribution of compound along axon ($P(x)dx = (1/(4\pi Dt)^{1/2})*e^{-x^2/4Dt}\,dx$)and you can calculate the amount of labeled aminoacids by using tables of normal (Gaussian) distribution or manually by using approximation of Gaussian integral: http://ece-www.colorado.edu/~bart/book/gaussian.htm
   1.5 days or 1.3×10^6 sec x 10^{-7} cm^2/sec gives 0.13 cm^2 or ±0.36 cm
   7 days or 6.1×10^6 sec x 10^{-7} cm^2/sec gives 0.61 cm^2 or ±0.78 cm

3) $\langle \Delta X^2 \rangle = 2Dt + (vt)^2$ The fact that protein is moved by fast axonal transport 1% of time with v=200mm/day mathematically reflected in effective v=2mm/day.

Lecture #4

1. Helicases put torsional energy into DNA that is relieved by topoisomerase 1. If we assume that the helicases create a torsional force, then how would you convert that force into a signal recognized by the topoisomerase.

Answer:
Any hypothesis are good as soon as they suggest at least one physically possible way to check tension in DNA strand.

2. Assuming that histone complexes neutralize 140 bp and are spaced on average every 200 bp and that the whole human genome is replicated in eight hours, what is the average rate of histone synthesis? What is the concentration of negative charges in the replicated nucleus (7 microns in diameter)?

Answer:
nucleosome complex = 8 histones = 140 bp

$Rate_{\text{histone synthesis}} = 1\ {}^{\text{genome}}/_{8\text{ hr}} * 6 \times 10^{9}\ {}^{\text{bp}}/_{\text{genome}} * 1\ {}^{\text{nucleosome complex}}/_{200\ \text{bp}} * 8\ {}^{\text{histones}}/_{\text{nucleosome complex}}$

$Rate_{\text{histone synthesis}} = 3 \times 10^{7}\ {}^{\text{histones}}/_{\text{hr}}$

For every 200 bp, 140 bp have their charges neutralized by the histones. So: 200 bp – 140 bp = 60 bp remaining with negative charges per nucleosome complex. There are 2 negative charges for every single base pair.

$V_{\text{nucleus}} = {}^{4}/_{3}\ pr^3 = = {}^{4}/_{3}\ p(7 \times 10^{-6}\ m/2)^3 = 1.8 \times 10^{-16}\ m^3 * 1000\ {}^{\text{Liters}}/_{m\text{\^{}}3} = 1.8 \times 10^{-13}\ L$

$\text{Concentration} = 6*10^{9}\text{bp in genome /200bp per histone} * 60\ {}^{\text{charged bp}}/_{\text{nucleosome complex}} * 2\ {}^{\text{negative charges}}/_{\text{charged bp}} * 1\ {}^{\text{mol negative charges}}/_{6.02 \times 10\text{\^{}}23\ \text{negative charges}} * 1/1.8 \times 10^{-13}\ L$

$Concentration_{\text{full synthesis}} = 0.033\ M$ negative charges

3. Describe a way to check for unpaired bases that involves interaction with the double stranded DNA and diffusion along it. Assuming a diffusion coefficient of $10^{-10}\ cm^2/sec$, how long a strand of DNA can be scanned in one hour (to get a reliable scan we suggest that you should only consider half the normal diffusion distance, i.e. 2X sampling).

Answer:

Distance of reliable scanning is a half of the diffusion distance $\langle \Delta X^2 \rangle = 2D\ t$ (t=one hour), and $L = \sim 4.25*10^{-4}$cm (in one direction for bidirectional scanning), or ~15000b.p.

Lecture #5

1 1. Read the review on mitosis (Nicklas, 1997) and tell me which of the two models for the block to chromosome separation is physically possible. A) The first model suggests that the concentration of a small molecule has to be greater than 1 μm. We postulate that the small molecule is produced by all chromosomes under tension (synthesis rate of X was 40,000 molecules/chromosome/minute) and degraded by chromosomes not under tension (degradation rate was given by the formula $d[X]/dt = -k[X]$, where $k = 10^{-4}\ s^{-1}$). The volume of the cell is 4000 μm^3 and there are 10 chromosomes. B) In the second model, another metabolite Y is being made by chromosomes under tension (the synthesis rate for Y is the same rate as for X above) and is remodeled by the chromosomes not under tension (synthesis rate is $d[Z]/dt = k_s[Y]$ where $k_s = 10^{-1}\ s^{-1}$) to produce the final produce Z which blocks chromosome separation. Z has a half- life of 10 minutes in cytoplasm and must be less than 10^{-7} M.

Nicklas, R.B. 1997. How cells get the right chromosomes. *Science*. 275:632-7.

A) In this model, comparing rates of synthesis and degradation:
$Rate_{synthesis} = 4 \times 10^4\ {}^{molecules}/_{chromosome \cdot min} * 1\ {}^{min}/_{60\ s} * 10$ chromosomes $* 1\ {}^{mol}/_{6.02 \times 10\text{^}23\ molecules} * 1/4000\ \mu m^3 * (1 \times 10^6\ \mu m)^3/1\ m^3 * 1\ m^3/1000\ L = 2.8 \times 10^{-9}\ {}^{M}/_{s}$

$Rate_{synthesis} = 2.8 \times 10^{-9}\ {}^{M}/_{s}$

$Rate_{degradation} = d[X]/dt = -k[X] = -10^{-4}\ s^{-1}\ [1 \times 10^{-6}\ M] = .01\ {}^{M}/_{s}$

$Rate_{degradation} = .01\ {}^{M}/_{s}$

Setting the maximum allowable concentration for degradation rate = concentration required for separation, we see that the degradation rate is much larger than the rate of synthesis at this maximum. If in any of the 10 chromosomes, there is a lack of tension, the molecule will basically be degraded to zero concentration almost immediately. Coordination of tension on all ten chromosomes simultaneously required for proceeding cell to division.

B) From above, $Rate_{synthesis} = 2.8 \times 10^{-9}\ {}^{M}/_{s}$, and
$Y_{concentration} = 2.8 \times 10^{-9}\ {}^{M}/_{s} * 10$ min $* 60$ s/1 min
$Y_{concentration} = 1.7 \times 10^{-6}$ M

$Rate_{remodeling} = d[Z]/dt = ks[Y] = 10^{-1}\ s^{-1}\ [1.7 \times 10^{-6}\ M] = 1.7 \times 10^{-7}\ {}^{M}/_{s}$
$Z_{concentration} = 1.7 \times 10^{-7}\ {}^{M}/_{s} * 10$ min $* 60$ s/1 min
$Z_{concentration} = 1.0 \times 10^{-4}$ M

Comparing the concentrations, synthesis and conversion rates, metabolite Y is converted to Z at a faster rate than it is actually produced. If at least one of ten chromosomes is not under tension, it will be converted to Z immediately. Plus, with a half-life of 10 minutes,

it degrades extremely slow compared to its synthesis rate, and will reach its concentration limit of 10^{-7} M after just:

$t = 10^{-7}\,M * 1/1.7 \times 10^{-7}\ {}^{M}/_{s}$
$t = 0.6$ s

After less than one second, the concentration of Z has exceeded its limit, which prevents division for quite long time required for degradation of Z, even though all chromosomes are under tension.

So, Model A is then more likely used by cells.

Lecture #6

1. To complete the replication of DNA in the normal S period of the cell cycle requires that multiple sites of DNA replication be initiated. For our average chromosome of 150 million bps, how many polymerases are needed to complete the replication in 8 hours with polymerases that move at 50 bp/s?
2. Estimates of the number of genes in the human genome are in the range of 50,000. If we assume that the coding region of each gene is 3,000 bp (corresponding to a molecular weight of 120,000 for an average protein—this is a little high), then how many replication events (cell divisions) are needed before a genetic mutation is generated on average in every gene (remember that there are 6,000,000,000 bp in the human genome) for the normal error frequency of 1 bp in 1,000,000,000 bp? Assume that the average volume of your cells is 3,000 microns cubed, then a 75 kg person will have approximately 2.5 x10^{13} cells that arose by binary division. How many mutant genes will the person have in at least one cell?

Answers:

1. Length of genome is $L=1.5*10^8$bp, and L should be replicated with the rate v=50bp/s in t=8h=28800s, so we need to begin replication in $N=1.5*10^8$bp/(v*t)=~104sites (and one site contains at least one molecule of polymerase)
2. Error frequency $f=10^{-9}$, multiplied by the total genome length $L=6*10^9$bp gives us m=6 mutations for one replication. Probability that this mutations will be in coding region of the gene is p=(50000genes*3000bp)/L=0.025, so we have m*p=~0.15 genes that will be mutant after each replication, and we need G=50000genes/(p*m)=~333333 replications for generation mutation in every gene. We can produce $N=2*10^{13}$ cells in $D=lg_2N$=~45 binary cell divisions. And each cell will contain at least M=m*D=~267 mutations, M*p=~7 of them will be in coding regions

Lecture #7

1. For every 200 bp of double stranded DNA, there is one nucleosome and nucleosomes bind with an effective binding constant greater than 10^9 M^{-1}.($\Delta G = -RT \ln Ka$, where R = 8.31 J/°K mole) Suppose that you want to screen all of the DNA of an average chromosome of 150 million base pairs. How many ATP molecules will be hydrolyzed in the screen, assuming that the energy of ATP hydrolysis is 29 kJ/mole. (see the notes for the treatment of the energy of binding)

2. If we have an mRNA that is needed at the level of ten copies per cell on average and the half- life of the mRNA in cytoplasm is ten minutes, then what fraction of the time should the gene be activated. Assume that the polymerase makes RNA at a rate of 20b/sec, that the original message is 6000 bases long, and that the next polymerase can start after the previous polymerase has moved 500 bp.

Answers:

1. Binding constant $K=10^9$ 1/M, so $E=\Delta G_{binding}=-RT\ln K=-12.4$kkal/mole-energy needed to unbind one nucleosome (for screening).

There are n=(150000000bp total)/(200bp per nucleosome)=750000 nucleosomes, and we need spend N=n*E/(7 Kcal/mole)=~15000000 molecules of ATP

2. Half life of 10 minutes means that 5 of 10 molecules will be degraded and system needs constant supply of 5 molecules every 10 minitas. L=6000bp, rate of synthesis is v=20bp/s, so time of synthesis is T=L/v=300s. Time between moment of transcription initiations t=500bp/v=25 s. 5^{th} copy of RNA will be start to be synthesized in 100^{th} second after beginning of synthesis of the first RNA molecule, and will finish synthesis in next T=300s, so cycle for synthesis of 5 molecules is t+T=400 s. And the gene should be activated 400s/10min=~66% of time.