BE/APh161: Physical Biology of the Cell
Homework 2
Due Date: Wednesday, January 18, 2012

"It would not be incorrect to say that their drills are battles without bloodshed, and the battles – bloody drills." - Josephus Flavius, book 3, chapter 5, line 75 of "The Jewish War against the Romans". This is the way that I think of doing our homework practice.

1. The Bleach-Chase Method.

In class we talked about the molecular census. Synthesis of new proteins is not the only kinetic process that governs how many proteins will be found in a given cell. An additional kinetic process of great importance is the decay of the proteins. In a recent paper (see the course website associated with this homework), the rate of protein decay was measured using a technique called "bleach-chase".

(A) Read the paper and write a one paragraph summary of what the paper is about, the essence of the method and the results. Note also that on all of the derivations that follow in this problem, you will be graded not only on having the right equations but also upon the clarity and logic of your presentation. Your job is to use the mathematics to explain how this method works and to explain precisely what is measured and how it is analyzed. This problem is an example of how we can take something right out of today's most very recent research results (paper from last year!) and turn it into a little story that you could explain to your scientifically-minded friends. Further, the question of how to actually go about measuring protein degradation rates is very hard and this paper shows us a very interesting answer to that question.

(B) To make the degradation process accessible, these cells have a fluorescent protein fused to some protein of interest whose degradation rate is the subject of enquiry. (NOTE: one of the things that they worried about and we should worry about too as readers is that maybe the act of tethering a big fluorescent protein to the protein of interest would alter its degradation rate, the very thing we are trying to measure.) In the absence of any

photobleaching, the evolution of the total number of fluorescent proteins, N_f follows the simple dynamical equation

$$\frac{dN_f}{dt} = \beta - \alpha N_f, \tag{1}$$

which acknowledges a rate of protein production β and a degradation rate α. Explain what this equation means and solve it as a function of time assuming that the initial number of proteins is zero. What is the steady-state value of the number of fluorescent proteins per cell? What is the characteristic time scale to reach this steady state in terms of parameters α and β?

(C) The problem of trying to infer the degradation rate from just looking at the amount of fluorescent protein is, however, that its dynamics is dictated not only by the degradation rate, but also by the production rate. The question is: can we create an experimental condition where we measure a quantity that is solely determined by the degradation rate? The experimental idea is that we have two populations of identical cells. What this means is that they have the same genomes, have been subjected to the same growth conditions and same environmental stimuli. In practice, what this really means is that the average fluorescence intensity of our protein of interest in the two populations is the same. At a certain instant in time, we then photobleach one of the two populations so that there fluorescent intensity is now reduced relative to its initial value and relative to the value in the unphotobleached population. We now have two populations. First, we have the number of unbleached molecules, N_u. Second we have the number of bleached molecules, N_p with a conservation law which is that the total number of proteins of our species of interest is given by $N_u + N_p$.

A similar equation to eqn 1 describes the dynamics of the unphotobleached molecules in the cells that have been subjected to photobleaching, with the number that that are unphotobleached given by N_u and described by the dynamical equation

$$\frac{dN_u}{dt} = \beta - \alpha N_u. \tag{2}$$

On the other hand, the number of photobleached proteins are subject to a different dynamical evolution described by the equation

$$\frac{dN_p}{dt} = -\alpha N_p, \tag{3}$$

since all that happens to them over time is that they degrade. Explain why there are two populations of proteins within the photobleached cells and why these are the right equations.

(D) Notice that while the evolution of the unbleached population still depends on both the production and degradation rates the time evolution of the bleached species is only dependent on the degradation rate. As a result, if we could track the amount of unbleached molecules as a function of time we would have direct access to α. The trick used by the authors in the paper is to evaluate the difference in the number of fluorescent proteins in the two populations. An absolutely critical assumption then is that

$$\frac{dN_f}{dt} = \frac{dN_u}{dt} + \frac{dN_p}{dt} \tag{4}$$

The point is that over time after photobleaching, the photobleached cells will become more fluorescent again as new fluorescent proteins are synthesized.

The key idea here is then to plot the difference between the intensity of the cells that were not disturbed by photobleaching and those that were. In particular, show the simple result that

$$\frac{d(N_f - N_u)}{dt} = -\alpha(N_f - N_u). \tag{5}$$

Note that this quantity is directly experimentally accessible since it calls on us to measure the level of fluorescence in the two populations and to examine the difference between them. Further, note that the dynamics depends strictly only upon the one parameter that we are trying to measure. Integrate this equation and show how the result can be used to determine the constant α that characterizes the dynamics of protein decay. Explain what you would actually plot if you were making the measurements and how that would yield the parameter α.

(E) In the paper, they discuss both degradation and dilution. Explain the distinction between these two ideas. Comment on which you think is dominant in bacteria based on what you learned from this paper.

2. Statistics of molecular partitioning.

In class I did a little demo to illustrate how binomial partitioning works conceptually with the idea that molecules subject to passive partitioning can be thought of as doing a coin flip to decide which daughter they will go to. In this problem, you will do the math of the problem by exploiting the binomial distribution.

(A) Assuming that the coin flip is *unfair* with probability of heads p and probability of tails q, show that the number of molecules n_1 going to daughter 1 when there are a total of N molecules of a given type in the mother cell is given by

$$p(n_1, N) = \frac{N!}{n_1!(N-n_1)!} p^{n_1} q^{N-n_1}. \tag{6}$$

(B) Show that the average number of molecules that go to daughter 1 is given by

$$\langle n_1 \rangle = \sum_{n_1=0}^{N} n_1 \frac{N!}{n_1!(N-n_1)!} p^{n_1} q^{N-n_1}. \tag{7}$$

Work the result out explicitly by using the observation

$$\langle n_1 \rangle = p \frac{\partial}{\partial p} \sum_{n_1=0}^{N} \frac{N!}{n_1!(N-n_1)!} p^{n_1} q^{N-n_1}, \tag{8}$$

and reconcile the result with the very intuitive way you would explain this to someone without doing a bunch of calculations. Similarly, show that the second moment of the distribution is given by

$$\langle n_1^2 \rangle = p \frac{\partial}{\partial p} p \frac{\partial}{\partial p} \sum_{n_1=0}^{N} \frac{N!}{n_1!(N-n_1)!} p^{n_1} q^{N-n_1} \tag{9}$$

and work out the result explicitly and show that this gives us

$$\langle n_1^2 \rangle - \langle n_1 \rangle^2 = Npq. \tag{10}$$

(C) We are interested in finding a measure of the variability from one cell to the next. Work out the ratio of the standard deviation to the mean

$$\frac{\sqrt{\langle n_1^2 \rangle - \langle n_1 \rangle^2}}{\langle n_1 \rangle} = \frac{1}{\sqrt{N}}. \tag{11}$$

Explain the significance of this result for the molecular partitioning during cell division. If $N = 10$, what kind of cell-to-cell variability do we expect? What about when $N = 100$?