Supplemental Materials

Molecular Biology of the Cell

Wheeler et al.

Appendix

Cell division by binary fission

The function relating proportion of cells observed in a particular state, and the time taken for that cell cycle stage, can be derived from the properties of exponential growth.

In a population undergoing exponential growth, the rate of population increase is proportional to the current population size, $N(t)$:

$$\frac{\partial N(t)}{\partial t} = \alpha N(t)$$

Where α is the growth constant. Solving this differential equation yields:

$$N(t) = N(0)e^{\alpha t}$$

Where $N(0)$ is the starting population size.

The population increase is driven by binary fission events so the rate of division events is the rate of population increase. As both daughter cells return to the start of the cell cycle after division the rate that cells return to the start of the cell cycle, $\partial F(t)/\partial t$, is twice that of division:

$$\frac{\partial F(t)}{\partial t} = 2\frac{\partial N(t)}{\partial t}$$

At any time, t, the rate at which cells are reaching time τ through the cell cycle is equal to the rate that cells returned to the start of the cell cycle at $t - \tau$:

$$\frac{\partial F(t-\tau)}{\partial t} = 2\frac{\partial N(t-\tau)}{\partial t} = 2\alpha N(t-\tau) = 2\alpha N(0)e^{\alpha(t-\tau)} = 2\alpha N(t)e^{-\tau\alpha}$$

The rate $\partial F(t-\tau)/\partial t$ cannot be experimentally determined from a fixed sample of cells. However $F(0 \le \tau \le n)$, the number of cells between the start of the cell cycle and a time of experimental interest, n, through the cell cycle, can be experimentally determined. We therefore consider the following integral:

$$F(0 \le \tau \le n) = \int_0^n \frac{\partial F(t-\tau)}{\partial t} d\tau = \int_0^n 2\alpha N(t)e^{-\tau\alpha}\, d\tau = 2N(t) - 2N(t)e^{-\alpha n}$$

The proportion of the total cell population $p(0 \le \tau \le n)$ at cell cycle stages between the start of the cell cycle and n is a more convenient experimental measure than the absolute number of cells $F(0 \le \tau \le n)$ which is a function of $N(t)$:

$$p(0 \le \tau \le n) = \frac{F(0 \le \tau \le n)}{N(t)}$$

Substituting $p(0 \le \tau \le n)$ to eliminate $N(t)$ yields:

$$p(0 \le \tau \le n) = 2 - 2e^{-\alpha n}$$

This may be rearranged to give:

$$n = -\frac{\ln\left(1 - \frac{p(0 \leq \tau \leq n)}{2}\right)}{\alpha}$$

For a population undergoing growth by binary fission the population doubling time is equal to the cell cycle length. The growth constant, α, is related to the length of the cell cycle, T:

$$N(T) = 2N(0)$$

$$2N(0) = N(0)e^{\alpha t}$$

$$\alpha = \frac{\ln(2)}{T}$$

Finally, substituting α yields [equation 2]:

$$n = -T\frac{\ln\left(1 - \frac{p(0 \leq \tau \leq n)}{2}\right)}{\ln(2)} = T - T\frac{\ln\left(2 - p(0 \leq \tau \leq n)\right)}{\ln(2)}$$

For a count of k cells (on the condition that $kp(0 \leq \tau \leq n) \geq 5$ and $k\left(1 - p(0 \leq \tau \leq n)\right) \geq 5$), the standard error of proportion is:

$$\Delta p(0 \leq \tau \leq n) = \sqrt{p(0 \leq \tau \leq n)\left(1 - p(0 \leq \tau \leq n)\right)/k}$$

The standard error in calculating time in any particular stage can be estimated from the error in determining the proportion of the population in stages up to and including n. Therefore, for a count of k cells:

$$\Delta t(0 \leq \tau \leq n) = t(0 \leq \tau \leq n) - T\frac{\ln\left(1 - \frac{p(0 \leq \tau \leq n) \pm \sqrt{p(0 \leq \tau \leq n)\left(1 - p(0 \leq \tau \leq n)\right)/k}}{2}\right)}{\ln(2)}$$

Cell division by multiple fission (schizogony)

Derivation of the function relating proportion of cells observed in a particular state to time spent in that state for cells undergoing fission into an arbitrary number of daughter cells is similar to that described in Cell division by binary fission. In binary fission two daughter cells are generated by a division event and the population increases by one. In the case of fission into arbitrary number of daughter cells the q daughter cells are generated by a division event and the population increases by $q - 1$.

The rate of population increase is proportional to the current population size, $N(t)$, and the population increase per division event:

$$\frac{\partial N(t)}{\partial t} = \alpha(q - 1)N(t)$$

Where α is the growth constant. Solving this differential equation yields:

$$N(t) = N(0)e^{\alpha(q-1)t}$$

Where $N(0)$ is the starting population size.

Upon each division event all (q) daughter cells return to the start of the division cycle. Therefore the rate that cells return to the start of the division cycle, $\partial F(t)/\partial t$, is:

$$\frac{\partial F(t)}{\partial t} = q\frac{\partial N(t)}{\partial t}$$

At any time, t, the rate at which cells are reaching time τ through the division cycle is equal to the rate that cells returned to the start of the cell cycle at $t - \tau$:

$$\frac{\partial F(t-\tau)}{\partial t} = q\frac{\partial N(t-\tau)}{\partial t} = q\alpha N(t-\tau) = q\alpha N(0)e^{\alpha(q-1)(t-\tau)} = q\alpha N(t)e^{-\tau\alpha(q-1)}$$

To derive $F(0 \le \tau \le n)$, the number of cells between the start of the cell cycle and a time of experimental interest, n, through the cell cycle, we consider the following integral:

$$F(0 \le \tau \le n) = \int_0^n \frac{\partial F(t-\tau)}{\partial t} d\tau = \int_0^n q\alpha N(t)e^{-\tau\alpha(q-1)}\, d\tau = \frac{qN(t)}{q-1} - \frac{qN(t)e^{-\alpha n(q-1)}}{q-1}$$

Consider $p(0 \le \tau \le n)$ to eliminate $N(t)$:

$$p(0 \le \tau \le n) = \frac{F(0 \le \tau \le n)}{N(t)}$$

$$p(0 \le \tau \le n) = \frac{q}{q-1} - \frac{qe^{-\alpha n}}{q-1}$$

This may be rearranged to:

$$p(0 \le \tau \le n)(q-1) = q - e^{\ln(q) - \alpha n}$$

$$n = \frac{\ln(q) - \ln\big(q - p(0 \le \tau \le n)(q-1)\big)}{\alpha}$$

For a population undergoing growth by fission into q parts the time taken for a population to increase by a factor of q is equal to the cell division length. The growth constant, α, is related to the length of the division cycle, T:

$$N(T) = qN(0)$$

$$qN(0) = N(0)e^{\alpha t}$$

$$\alpha = \frac{\ln(q)}{T}$$

Substituting α yields [equation 3]:

$$n = T\frac{\ln(q) - \ln\big(q - p(0 \le \tau \le n)(q-1)\big)}{\ln(q)} = T - T\frac{\ln\big(q - p(0 \le \tau \le n)(q-1)\big)}{\ln(q)}$$

Cellular senescence

The expected proportion of cells in each generational age can be calculated using the same principles as Cell division by binary fission, however here we are assuming the mother and daughter cell of any division can be distinguished instead of viewing both the mother and daughter cell as identical and returning to the start of the cell cycle.

At any time, t, the number of cells which are the age τ are the daughter progeny of cell division events which occurred at $t - \tau$. They were therefore generated at a rate, $\partial F(t-\tau)/\partial t$, equal to the rate that cells reached division at $t - \tau$:

$$\frac{\partial F(t-\tau)}{\partial t} = \frac{\partial N(t-\tau)}{\partial t} = \alpha N(t-\tau) = \alpha N(0)e^{\alpha(t-\tau)} = \alpha N(t)e^{-\tau\alpha}$$

The number of cells, $G(n)$, at a particular generational age, n, is the number of cells generated in the time interval from $T(n-1)$ to Tn, where T is the length of the cell cycle:

$$G(n) = F(T(n-1) \leq \tau \leq Tn) = \int_{T(n-1)}^{Tn} \frac{F(t-\tau)}{\partial t} d\tau = N(t)e^{-\alpha T(n-1)} - N(t)e^{-\alpha Tn}$$

As

$$\alpha = \frac{\ln(2)}{T}$$

$$G(n) = N(t)2^{1-n} - N(t)2^{-n} = N(t)2^{-n}$$

To eliminate $N(t)$, consider the proportion of the total cell population, $p(n)$, which are in generation n:

$$p(n) = \frac{G(n)}{N(t)}$$

Substituting $p(n)$ and eliminating $N(t)$ yields:

$$p(n) = 2^{-n}$$

Continuous variables

This transition from considering a discrete set of sub-categories based on a continuous variable, to analysis treating that variable as continuous can be considered mathematically. Let $p(l_n)$ represent the proportion of cells in each category l, of which there are n. The cumulative distribution equivalent to $p(l_n)$ is:

$$P(l_m) = \sum_{n=0}^{m} p(l_n)$$

Where m is the number of categories (number of values for which $p(l_n)$ is defined). In the limit $m \to \infty$:

$$P(l) = \int_{0}^{l} p(r)\, dr$$

In this limit $p(r)$ is an estimation of the continuous probability distribution which describes the distribution of cells through the cell cycle relative to an arbitrary criterion of cell cycle stage, r.

The distribution of cells through the cell cycle relative r can be determined by several methods, see main text.