Balanced Growth, Pools, and Turnover

Adapted and derived from A.P. Sims & B.F. Folkes, "A kinetic study of the assimilation of [15N]-ammonia and the synthesis of amino acids in an exponentially growing culture of Candida albicans", *Proc. Royal Soc. B, 479-502 (1964).*

1) Exponential Growth

A bacterial culture that is not limited by nutrients grows exponentially, where the number of cells in the culture increases at a fixed ratio per unit time. The differential equation that describes exponential growth is:

Eqn 1) $$\frac{dQ}{dt} = kQ$$

where Q is the number of cells, k is the growth rate constant (s^{-1}), and dQ/dt is the rate of change in the number of cells (cells/s). This differential equation can be solved to provide an expression for the number of cells at any given time:

$$\frac{dQ}{Q} = k\,dt$$
$$\int \frac{dQ}{Q} = \int k\,dt$$
$$\ln Q\Big|_{Q_0}^{Q(t)} = kt\Big|_0^t$$
$$\ln\left(\frac{Q(t)}{Q_0}\right) = kt$$
$$\frac{Q(t)}{Q_0} = \exp(kt)$$
$$Q(t) = Q_0 \exp(kt)$$

Eqn 2) $$Q(t) = Q_0 \exp(kt)$$

where Q_0 is the number of cells present at t=0. This expression for exponential growth can be used to calculate the "doubling time" in terms of the growth rate constant k:

Eqn 3)
$$Q(t_2) = 2Q_0 = Q_0 \exp(kt_2)$$
$$\ln 2 = kt_2$$
$$t_2 = \frac{\ln 2}{k} = \frac{0.693}{k}$$

A doubling time of 40 minutes corresponds to a growth rate constant k = 2.89e-4 s^{-1}.

2) Balanced Growth

The principle of balanced growth refers to the fact that the average composition of the cells in an exponentially growing culture does not change. There is a certain amount of each metabolite, protein, or nucleic acid species in each cell, and since the number of cells is growing exponentially, the amount of each individual species is also growing exponentially.

Thus, for any species X in the cell, the differential equation that describes the amount of that species in the culture is given by:

Eqn 4) $$\frac{dX}{dt} = kX$$

This condition provides an important constraint when dealing with steady state concentrations of metabolites and intermediates.

3) Synthesis and Utilization

Any given component of the cell is synthesized at a certain rate and utilized at a certain rate. The steady state concentration of each metabolite represents the balance between these two dynamic processes. For a given metabolic intermediate M, the synthesis rate and utilization rate are represented by the following mechanistic flows:

Eqn 5) $$\xrightarrow{k_s} M \xrightarrow{k_u}$$

where k_s is the synthesis rate constant (molecules/cell-s) and k_u is the utilization rate constant (molecules/cell-s). The differential equation that describes the rate of change of the amount of M in the culture is given by:

Eqn 6) $$\frac{dM}{dt} = k_s Q - k_u Q$$

The synthesis and utilization rate constants are aggregate rate constants that take into account synthesis or utilization by all possible pathways. The amount of M in the cell is clearly determined by the relative magnitudes of these two rates.

4) Pool Size

The pool size (P) of a metabolite is the number of molecules per cell, given by

Eqn 7) $$P = \frac{M}{Q}$$

The magnitude of the pool can be determined by combining the requirements for synthesis of M (Eqn 4) to meet balanced growth with the synthesis and utilization rates (Eqn 6):

Eqn 8) $$\frac{dM}{dt} = kM = k_s Q - k_u Q$$
$$\frac{M}{Q} = \frac{k_s - k_u}{k} = P$$

Thus, the pool size P is directly determined by the magnitudes of the synthesis, utilization, and growth rate constants. This equation can be rearranged to permit an intuitive interpretation of the balance of metabolic flows:

Eqn 9) $k_s = k_u + kP$

which reads “the synthesis rate equals the utilization rate plus the requirements for pool replacement”. As the cell grows, M may be used for many purposes, but the overall synthesis rate must exceed these requirements by an amount that allows the pool of M to double every generation time.

5) Pool Turnover

The flux through a metabolic pool of a given size may be either fast or slow depending on the magnitudes of the synthesis and utilization rate constants. The turnover of the pool is the number of times per generation that the pool is replaced during balanced growth. The rate of synthesis of a metabolite is given by:

Eqn 10) $\frac{dM}{dt} = k_s Q$

and the total amount of M that is synthesized in a generation (M_2) is computed by integrating this equation over one doubling time. The size of the metabolite pool prior to doubling is given by M_0, and the number of times the pool M_0 is synthesized in one generation by the ratio of M_2 to M_0. This quantity is the turnover number T, which is the number of times the pool of M turns over in one generation of balanced growth.

$$M_2 = \int_0^{t_2} k_s Q(t)dt = \int_0^{t_2} k_s Q_0 \exp(kt)dt$$

$$M_2 = \frac{k_s}{k} Q_0 \exp(kt)\Big|_0^{t2} = \frac{k_s}{k} Q_0 (\exp(kt_2) - 1)$$

$$M_2 = \frac{k_s}{k} Q_0$$

$$\frac{M_0}{Q_0} = \frac{k_s - k_u}{k} = P$$

$$M_0 = \frac{k_s - k_u}{k} Q_0$$

$$\frac{M_2}{M_0} = \frac{\frac{k_s}{k} Q_0}{\frac{k_s - k_u}{k} Q_0}$$

$$\frac{M_2}{M_0} = \frac{k_s}{k_s - k_u}$$

Eqn 11) $T = \frac{M_2}{M_0} = \frac{k_s}{k_s - k_u}$

6) Relationships between Pool Size, Turnover, Synthesis and Degradation Rate Constants

The steady state metabolite pool during balanced growth is determined by the magnitudes of the synthesis and degradation rate constants. The pool size and turnover number are defined in terms of these constants and the overall growth rate constants. These two views of a metabolite pool can be readily interconverted.

Eqn 12) $P = \frac{k_s - k_u}{k}$

The pool size is determined by the amount the synthesis rate exceeds the utilization rate, normalized for the growth rate.

Eqn 13) $$T = \frac{k_s}{k_s - k_u}$$

The turnover number is the ratio of the overall synthesis rate to the difference between the synthesis and degradation rates.

The equations for P and T can be solved for values of k_s and k_u:

Eqn 14) $$k_s = kPT$$

The overall metabolite synthesis rate is given by the growth rate times the pool size times the number of times the pool turns over per generation.

Eqn 15) $$k_u = kPT - kP = k_s - kP$$

The overall metabolite utilization rate is given by the difference between the overall synthesis rate and the rate of synthesis required to replace the metabolic pool.

7) Relationship between Turnover Number and Pulse Labeling Experiments

In a pulse labeling experiment, the progress of the label through the metabolite pool is monitored. The specific activity of the label entering the pool is given by θ_0, and the specific activity of the pool of M is given by θ.

Eqn 16) $$\overset{\theta_0}{} \xrightarrow{k_s} \overset{\theta}{M} \xrightarrow{k_u}$$

The total amount of M at any time is the sum of the labeled and unlabeled amounts of M:

Eqn 17) $$M_T = M + M^*$$ $$\theta = \frac{M^*}{M_T}$$

The kinetic equations that governs the flow of labeled and unlabeled M in and out of the pool are given by:

$$\frac{dM}{dt} = k_s Q(1-\theta_0) - k_u Q(1-\theta)$$

Eqn 18) $$\frac{dM^*}{dt} = k_s Q\theta_0 - k_u Q\theta$$

$$\frac{dM_T}{dt} = \frac{dM}{dt} + \frac{dM^*}{dt} = k_s Q - k_u Q$$

In a pulse labeling experiment, the specific activity of the pool of M is monitored as a function of time. Combining Eqn 17 and 18 gives the rate at which the specific activity changes:

Eqn 19) $$\frac{d\theta}{dt} = (\theta_0 - \theta)kT$$

$$\frac{d\theta}{dt} = \frac{d\left(\frac{M^*}{M_T}\right)}{dt} = \frac{\left(M_T \frac{dM^*}{dt} - M^* \frac{dM_T}{dt}\right)}{{M_T}^2}$$

$$\frac{d\theta}{dt} = \frac{\left(\frac{dM^*}{dt} - \theta \frac{dM_T}{dt}\right)}{M_T}$$

$$\frac{d\theta}{dt} = \frac{k_s Q\theta_0 - k_u Q\theta - \theta(k_s Q - k_u Q)}{M_T}$$

$$\frac{d\theta}{dt} = k_s(\theta_0 - \theta)\frac{Q}{M_T} = (\theta_0 - \theta)\frac{k_s}{P}$$

$$\frac{d\theta}{dt} = (\theta_0 - \theta)\frac{k_s k}{k_s - k_u}$$

$$\frac{d\theta}{dt} = (\theta_0 - \theta)kT$$

This equation can be integrated to solve for the time dependence of θ:

Eqn 20) $$\theta(t) = \theta_0(1 - \exp(-kTt))$$

So, the apparent rate constant for pulse labeling of the pool is simply the growth rate constant times the turnover number. This provides a key connection between the results from pulse labeling experiments and the turnover of the metabolic pool.

$$\frac{d\theta}{\theta_0 - \theta} = kT\,dt$$

$$\int \frac{d\theta}{\theta_0 - \theta} = \int kT\,dt$$

$$-\ln(\theta_0 - \theta)\Big|_0^{\theta(t)} = kTt\Big|_0^t$$

$$\ln\left(\frac{\theta_0 - \theta(t)}{\theta_0}\right) = -kTt$$

$$\frac{\theta_0 - \theta(t)}{\theta_0} = \exp(-kTt)$$

$$\theta(t) = \theta_0(1 - \exp(-kTt))$$