Cell Growth and Size Homeostasis in Proliferating Animal Cells
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Experimental Question

• Is there an intrinsic mechanism for coordinating growth and cell cycle in metazoan cells?

• If so, what is this mechanism dependent on?
Background

• Two models:
  – Exponential growth
  – Linear growth

• Other systems
  – Budding Yeast – size dependent growth rate
  – Bacteria - unknown
Background

• Metazoan System
  – Unclear whether regulation exists
  – Regulation may be the result of separate growth and mitogenic signals from the environment
  – Conflicting studies

• Statistics
  – Need for synchronized populations
  – Cell Cycle Inhibitors
Background

• Purpose: Determination of the growth function for lymphoblastoid leukemia cells

• How: Combined a gentle cell synchronization technique with mathematical analysis
Measuring size dependency on growth in asynchronous populations

• Collin Richmond Method:
  – At steady state, # cells smaller than size S only increases when cells larger than size S divide and only decreases when cells smaller than size S grow
  – Proportion of cells of any size does not change in time, so two numbers must be equal

• Problem: Requires size distribution of newborn subpopulation and distribution of cells just before division. Difficult to obtain
Measuring size dependency on growth in asynchronous populations

- Subpopulation of newborns
  - Mouse lymphoblastoids on a membrane
  - Division
    - One daughter cell eluted
    - Other continues to divide

Unsynchronized Population

Newborn Population
Measuring size dependency on growth in asynchronous populations
Implementation of Collins-Richmond Method

\[ \nu(s) = 2\alpha \frac{F_0(s)}{f_a(s)} - \alpha \frac{(F_0 \ast \delta)(s)}{f_a(s)} - \alpha \frac{F_a(s)}{f_a(s)} \]

- \( \nu(s) \) = Cell Growth Rate
- \( f_a(s) \) = asynchronous size probability distribution
- \( F_a(s) \) = cumulative \( f_a(s) \)
- \( F_0(s) \) = newborn cumulative size distribution
- \( \delta(\Delta) \) = distribution of differences between newborns
- \( \alpha \) = fraction of dividing cells/minute

![Graph A](image1.png)
![Graph B](image2.png)
Conclusions from Collins-Richmond

• Larger cells have higher growth rates throughout most of cell size range.
• Beyond critical size (vol = 2000fl for L1210), trend is reversed, but 65% of population has already divided before reaching this size

But, C-R is inadequate because of growth rate heterogeneity within the population
Time dependency of growth

- Comparison of pairs of size distributions from synchronized populations at one hour intervals
- Average rate at which cells grow in each interval

\[ \beta_n = \frac{1}{N_t} \sum_{i=1}^{N_t} \beta^i_n \]
Time dependency of growth

\[ f_n(s) = \int_{c=0}^{\infty} f_0(s-c) \varphi_n(c) \, dc \]

Deconvolve to find \( \varphi_n(c) \)
then use that to find \( c_n \) for each time point

\[ c_n = \int c \varphi_n(c) \, dc = \langle c^i_j \rangle \]

Relate calculate values \( c_n \) to the mean growth rates \( \beta_n \)

\[ \beta_n = c_n - \sum_{j=0}^{n-1} \beta_j \Delta t \]

Because \( \Delta t = 1 \),

\[ \beta_n = c_n - \sum_{j=0}^{n-1} \beta_j \]

\( c_n \) = mean \( c \) value at \( t=n \)

\( \varphi_n(c) \) = probability distribution of \( c^i_n \)

\( f_n \) = measured distribution from time \( n \)
Time dependency of growth

- Mean linear growth constants for each time interval
- Distribution of cell cycle stages
- Mean exponential growth constants for each time interval
Time dependency of growth
Dependence of Cell Division on Time and Size

• Is there a size gate that shortens the cell cycle?
  – Examine Interval at which most cells divide (9-12 hours)
  – Use growth exponential constants and compare size distributions at consecutive time intervals
Dependence of Cell Division on Time and Size

• Calculate frequency of cell division as a function of cell cycle time
Dependence of Cell Division on Time and Size

• If cells were all same age, likelihood of division increased with increasing cell size.
• If cells were all same size, likelihood of division increased with increasing age.
• If $\psi =$ likelihood of division, $\tau =$ cell age, and $s =$ cell size, probability of division:

$$d\psi = \left(\frac{\partial \psi}{\partial \tau}\right)_s d\tau + \left(\frac{\partial \psi}{\partial s}\right)_\tau ds$$
Discussion

• Size of cell reflects the relationship of its growth rate and division frequency

• Accelerative growth phase in G\textsubscript{1} follow by stable exponential growth during the rest of the cell cycle

• Growth and division independently determined by size and age

• Very large cells (above threshold) behave differently than this model