A Cooperative Transition in Suspension Culture of Amoeba

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The Slow to Fast (Lag to Log) Proliferation Transition in Suspension Culture of Dictyostelium discoideum, in Vegetative / Unicellular State

Inoculation at low density

Doubling Time in Log Phase is 12 hours

Is the growth rate transition a sign of adaptation (conventional belief) or a collective growth effect? Predictive theory lacking.
Motivation to Study the Slow-Fast Transition

• Practical Interest: infection, microscale cell proliferation, cancer expansion

• Scientific Challenge: New Behavior at Low Density, a Tractable Regime: Obvious Mean Field Theory fails

\[ \dot{n} = \gamma n + \kappa n^2 + \ldots \]

where \( n \) is cell density,
expect exponential growth at low not high density, as observed

* What’s the mechanism?
Earlier Conclusion: Collective Proliferation Via Cell-Cell Collisions

Peclet Number = rate of transport by advection / diffusion

<table>
<thead>
<tr>
<th>Pe = u L /D</th>
<th>Doubling Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>9.9 ± 1.3</td>
</tr>
<tr>
<td>1.0</td>
<td>8.0 ± 1.0</td>
</tr>
<tr>
<td>10.0</td>
<td>8.9 ± 1.4</td>
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</tbody>
</table>

No sign of soluble growth factor (endocrine) signaling

Agreement with theory of signaling via cell-cell collisions

\[ \dot{n} = \gamma P_G(n) n \]

Improved Counting Technique: Extraordinary Variation

Reduced lagging does not propagate as a new strain.

Why is there such variation in growth behavior?
Additional Precautions Taken

- Test for possible contaminations: no effect on lagging seen with removal of antibiotics, use of new antibiotics
- Sterilizing lights added to clean table
- Room lights left on to suppress circadian rhythms
- Fresh strains obtained from Dicty Stock Center, selected for fastest log phase growth
- Many simultaneous growth curves measured
Improved Growth Curves

Transition and Variation Persists
Lagging According to Shaker (25 ml) and Vial (0.6 ml) Experiments

<table>
<thead>
<tr>
<th>Run</th>
<th>Number of Samples</th>
<th>Log Phase Doubling Time (hr)</th>
<th>Average Lag Time (hr) and Standard Deviation</th>
<th>Range of Lag Times (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Volume Shaker Run 1</td>
<td>5</td>
<td>11.3</td>
<td>28 ± 6</td>
<td>19 to 33</td>
</tr>
<tr>
<td>Large Volume Shaker Run 2</td>
<td>6</td>
<td>9.8</td>
<td>67 ± 9</td>
<td>59 to 85</td>
</tr>
<tr>
<td>Small Vial Run 1</td>
<td>15</td>
<td>11.4</td>
<td>41 ± 11</td>
<td>25 to 64</td>
</tr>
<tr>
<td>Small Vial Run 2</td>
<td>15</td>
<td>12.8</td>
<td>47 ± 24</td>
<td>19 to 95</td>
</tr>
</tbody>
</table>

Variation Confirmed
Combined Vial Results: Note Extraordinarily Long Lag Times

Lag Time (hours)

Frequency
Critical Test of Collision Theory: Variable Shear Rate Test

Laminar Flow System for vial culture for 16-64 rpm (vs. 150 rpm in shaker culture)

Cell-Cell Collision Theory Fails

Graph showing the density of cells/ml over time for different RPM settings.

- 32 rpm Data
- 64 rpm Data
- 16 rpm Data
- 32 rpm Theory
- 64 rpm Theory
- 16 rpm Theory
Signaling by Growth factors Transported by Diffusion and Advection?
Conditioned Media Experiment

This is a repeat of an earlier experiment with lower sensitivity that had given a null result (Phys. Rev. E 2008)
Conditioned Media Results

Noticeable Shift between 100% and other values Indicates Chemical Signaling Mediates Growth Transition
Endocrine Signaling Theory for Slow-Fast Transition

\( c \) = concentration of growth factor

\( n \) = cell density

Expect that in the lag phase: \( \dot{c} = \nu n \) therefore at transition

\[
c_x = \left( \nu \frac{n_0}{\gamma_{\text{slow}}} \right) \left( \exp(\gamma_{\text{slow}} t) - 1 \right)
\]

Estimate \( c_x \) at \( 6 \times 10^{-10} \) to \( 10^{-8} \) M and \( K_D \) as \( 8 \times 10^{-11} \) to \( 2 \times 10^{-8} \) M typically \( 5 \times 10^{-10} \) M

Then \( c_x/K_D \) is 0.03 to 170 most likely 1 to 28,

compared to saturation of EGF receptors at \( c_x/K_D = 1-2 \).

Conclusion: chemical signaling mechanism is plausible.
Candidate Explanations for Variation in Growth Curves

• Recall we observed variation in lag times as follows: standard deviations of 6 to 24 hours and ranges of variation extend to as much as 40 to 76 hours.

• Inoculation uncertainty: for vials, 50 cells imply lag variation of 2.4 hours, but for shakers, 2500 cells, expect 0.3 hours. This rules out shot noise in initial density.

• Variations due to initial phase in cell cycle. Estimate variation in lag time is comparable to doubling time in log phase, 12 hours. A possibility.
Growth Factor Receptor Binding
Fluctuation Theory for Variation in Lagging

- need to explain $\sigma_{\text{lag}}$ of 10 to 15 hours, which implies $\frac{\sigma_{n_x}}{n_x} = \nu_{\text{slow}} \sigma_{\text{lag}} = 0.40$ to 0.59

- Employ theory of fluctuation (Lauffenburger, 1993) in binding occupancy ($\theta$):
  \[ \sigma_{\theta} = (c_x K_D)^{\frac{1}{2}}/R^{1/2}(K_D + c) \]
  where $R$ is the average number of receptors per cell.

  We require $\frac{c_x}{K_D} < 1.4 \times 10^{-4}$ or $> 80$. Either seems implausible.
Conclusions and Speculations

• The lag-log proliferation transition in suspension culture of the model eukaryote *Dictyostelium discoideum* is a collective effect mediated by soluble growth factors.

• Variation in the growth curves is not due to fluctuations in receptor binding, possibly due to variations in cell cycle phase in inoculants.

• The (dis)appearance of very long lagging samples might indicate cells have spontaneously moved in and out of epigenetically different growth states.

• Growth on surfaces vs. suspension
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