A Cooperative Transition in Suspension Culture of Amoeba

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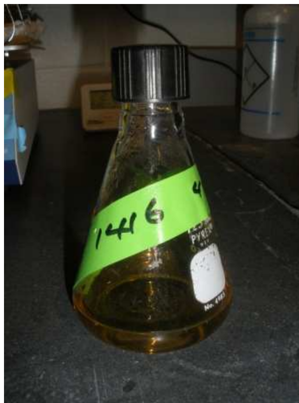
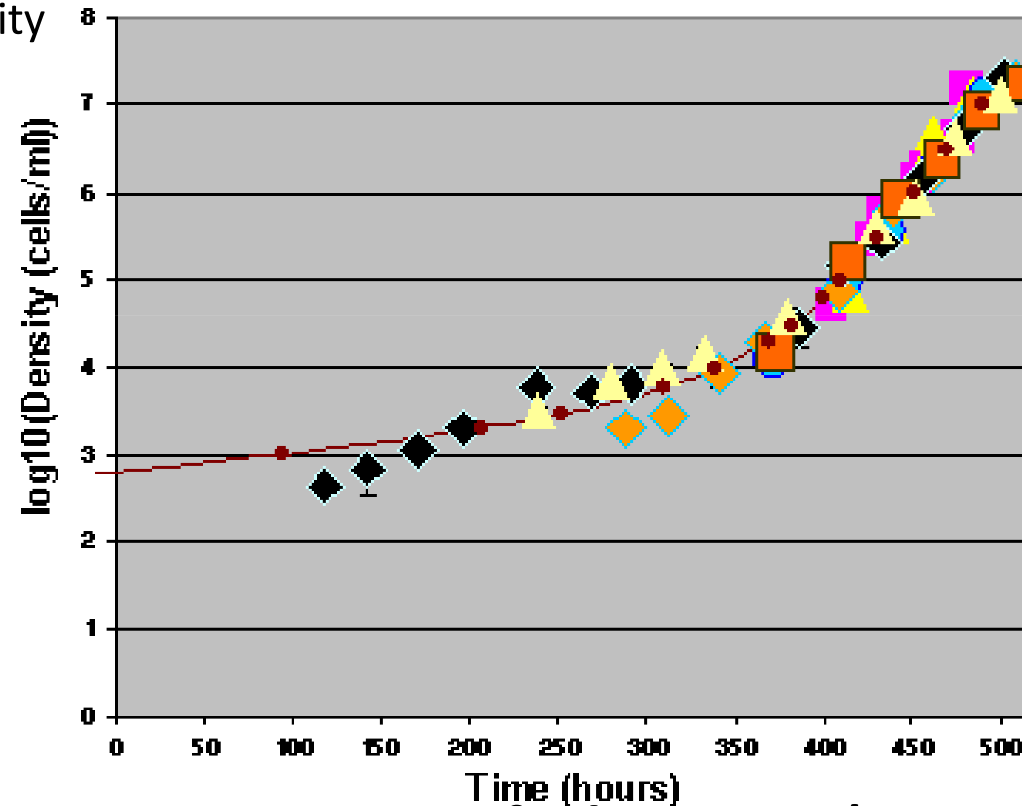
The Slow to Fast (Lag to Log) Proliferation Transition in Suspension Culture of *Dictyostelium discoideum*, in Vegetative / Unicellular State

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

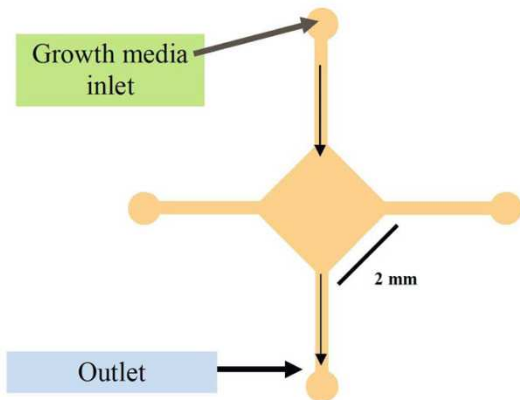
- Practical Interest: infection, microscale cell proliferation, cancer expansion
- Scientific Challenge: New Behavior at Low Density, a Tractable Regime: Obvious Mean Field Theory fails $\dot{n} = \gamma\, n + \kappa\, n^2 + \ \ldots$

  where n is cell density,

  expect exponential growth at low not high density, as observed

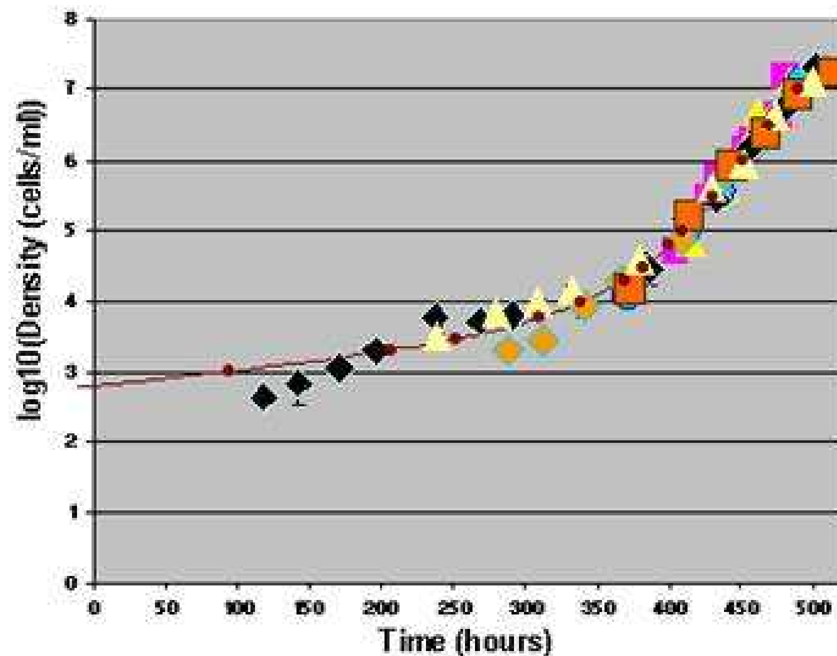
* What's the mechanism?

Earlier Conclusion: Collective Proliferation Via Cell-Cell Collisions



Peclet Number = rate of transport by advection / diffusion

Pe = u L /D	Doubling Time (hr)
0.4	9.9 ± 1.3
1.0	8.0 ± 1.0
10.0	8.9 ± 1.4



No sign of soluble growth factor (endocrine) signaling

Agreement with theory of signaling via cell-cell collisions

$$\dot{n} = \gamma \, P_G(n) \, n$$

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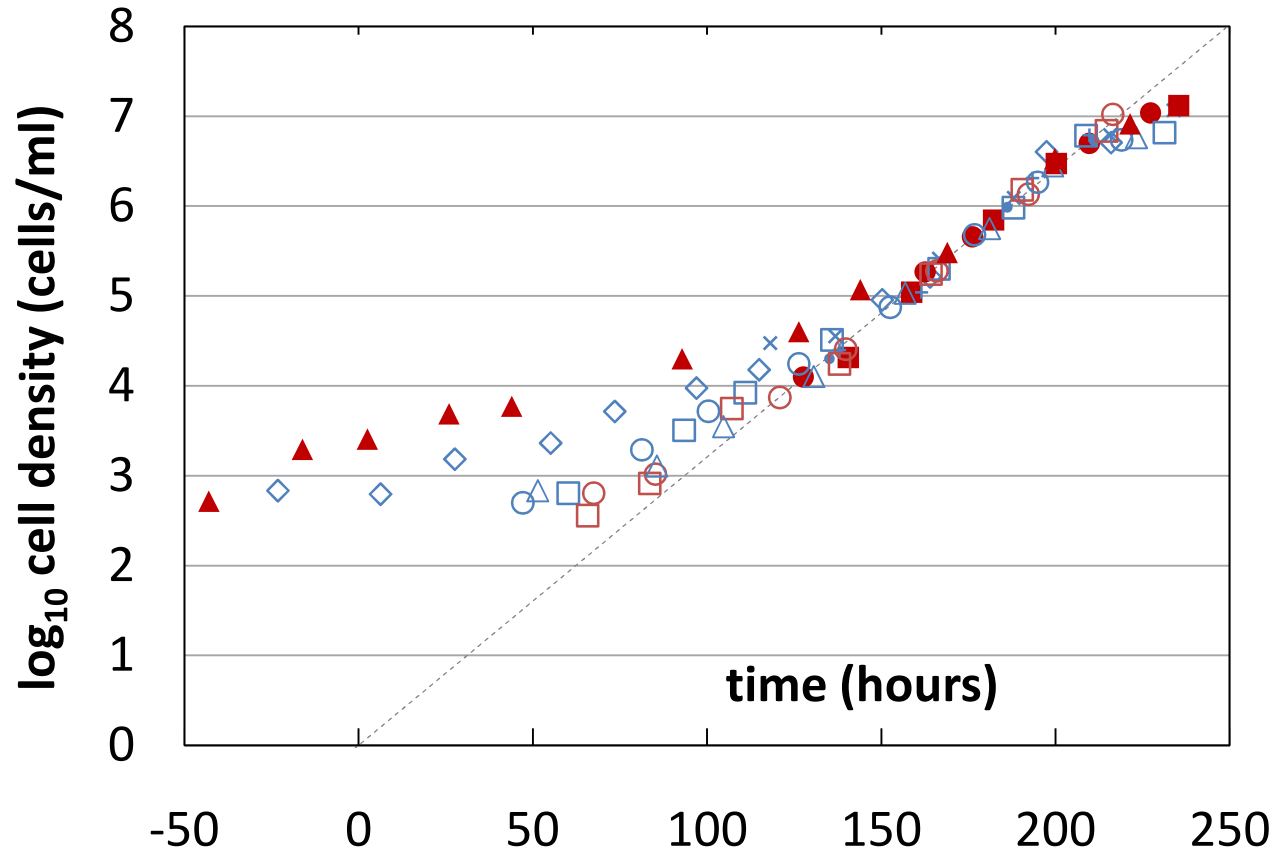
Improved Counting Technique: Extraordinary Variation



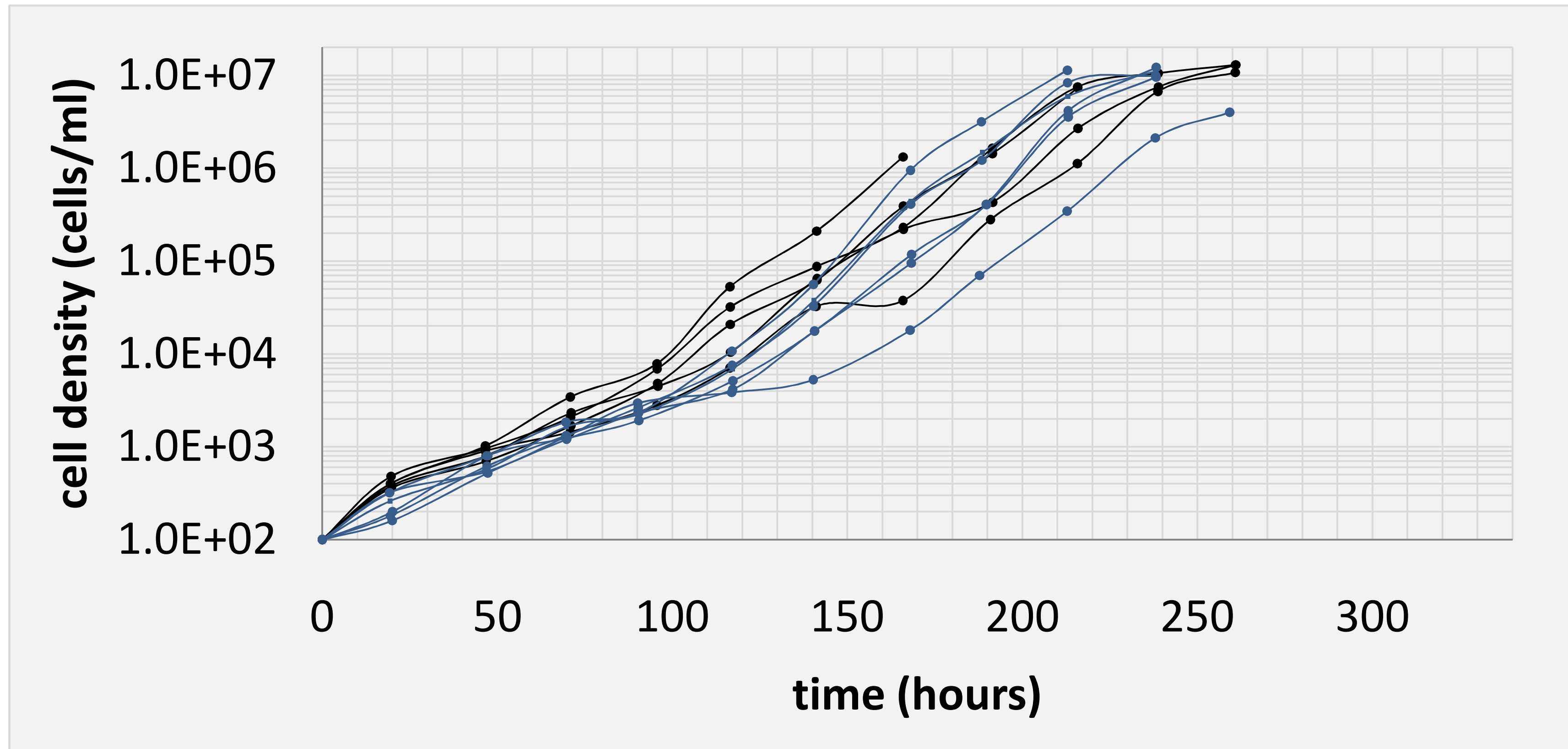
Reduced lagging does not propagate as a new strain.

Why is there such variation in growth behavior?

Additional Precautions Taken

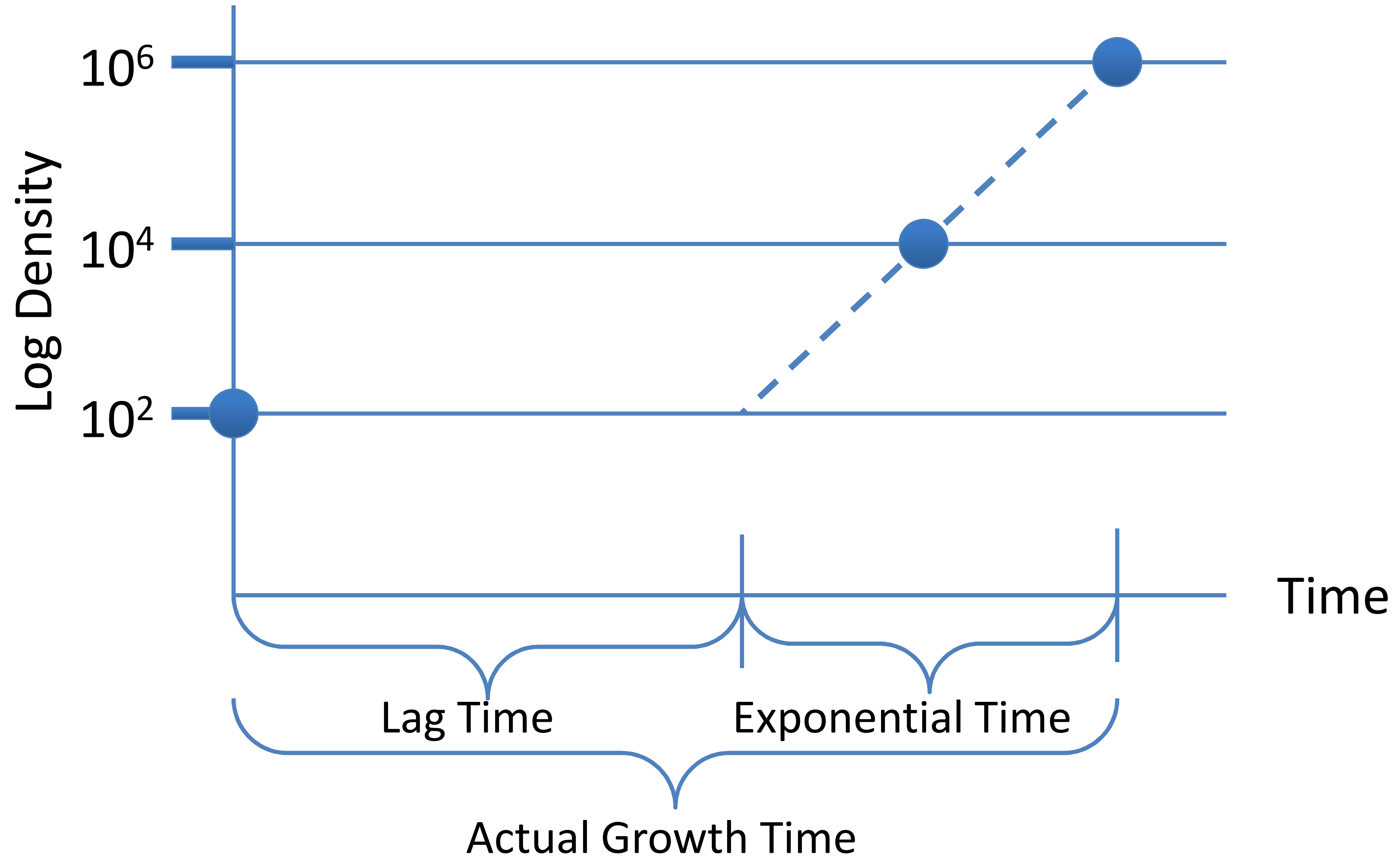
- Test for possible contaminations: no effect on lagging seen with removal of antibiotics, use of new antibiotics
- Sterilizing lights added to clean table
- Room lights left on to suppress circadian rhythms
- Fresh strains obtained from Dicty Stock Center, selected for fastest log phase growth
- Many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

Improved Growth Curves



Transition and Variation Persists

Closed Vial Experiment

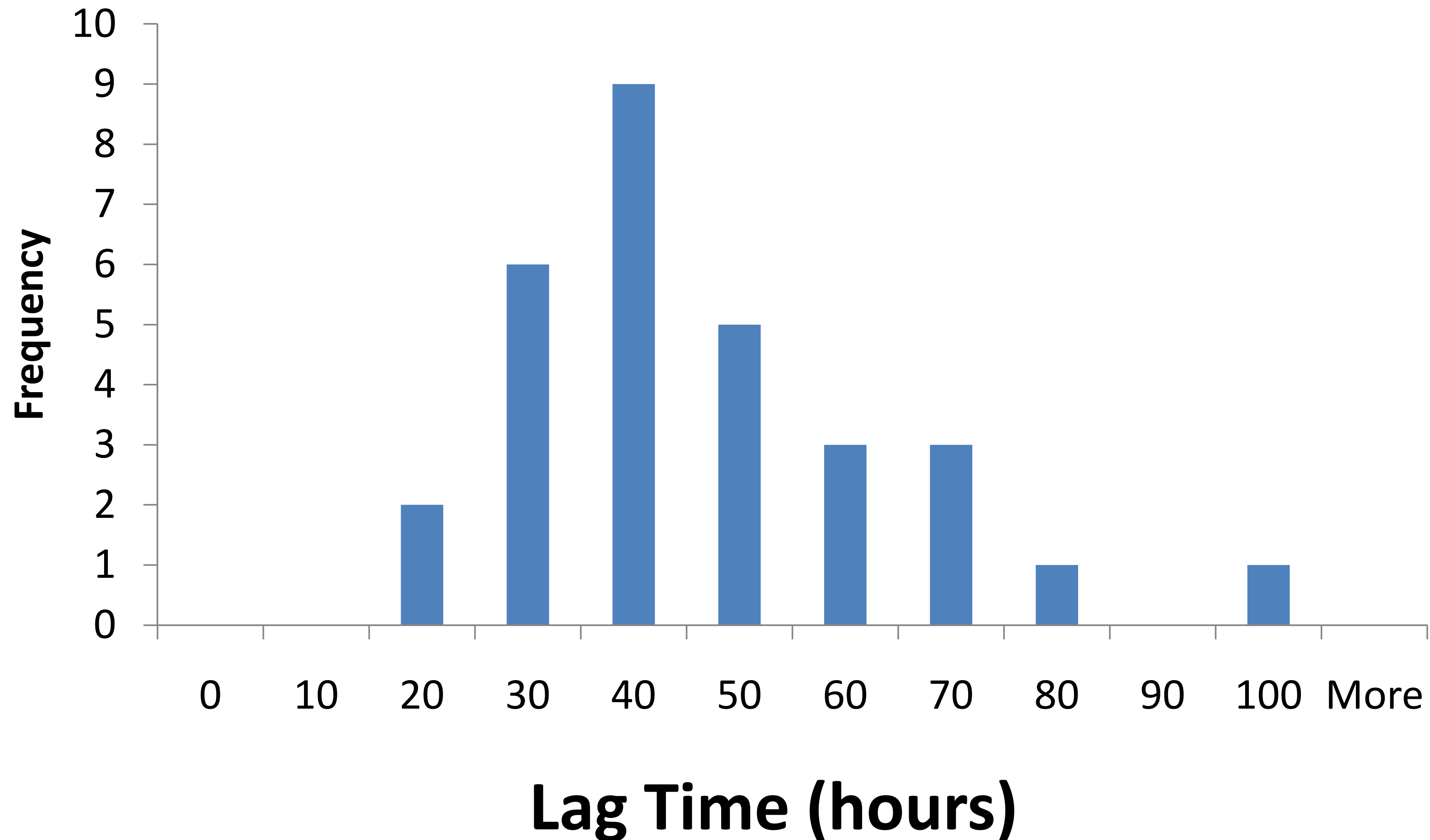


Lagging According to Shaker (25 ml) and Vial (0.6 ml) Experiments

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

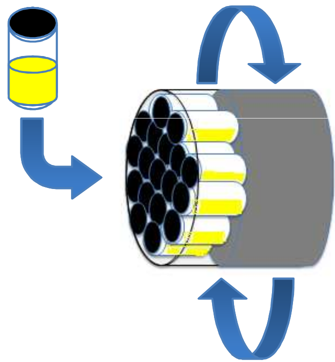
Variation Confirmed

Combined Vial Results: Note Extraordinarily Long Lag Times

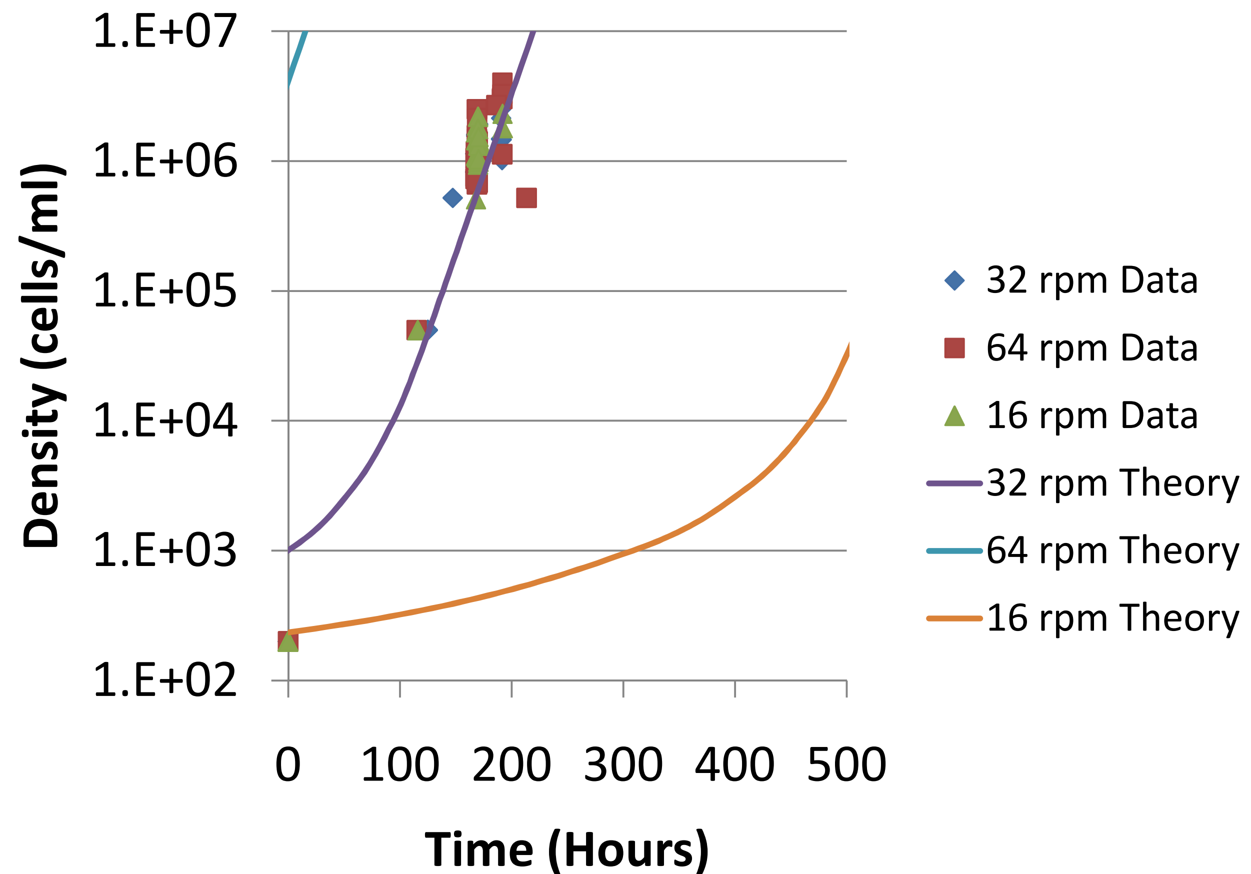


Critical Test of Collision Theory: Variable Shear Rate Test

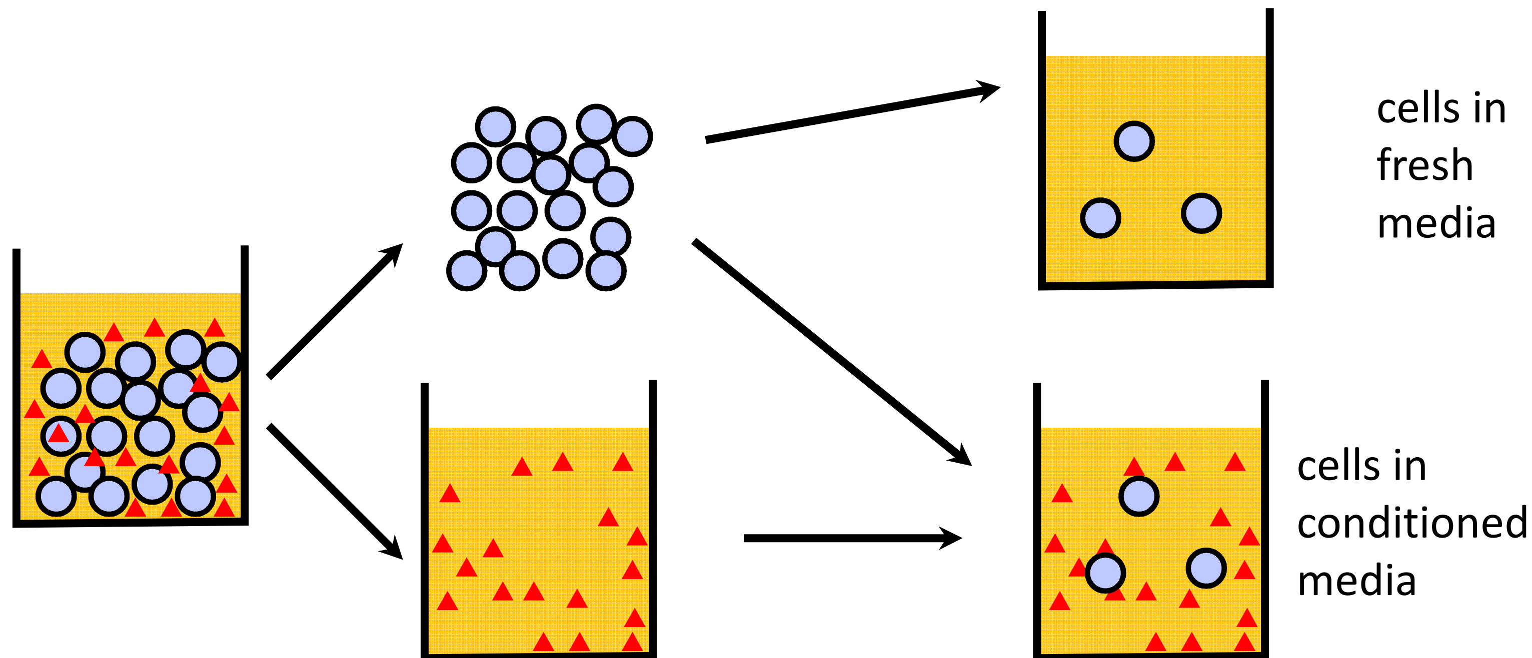
Laminar Flow System for vial culture for 16-64 rpm (vs. 150 rpm in shaker culture)



Cell-Cell Collision Theory Fails

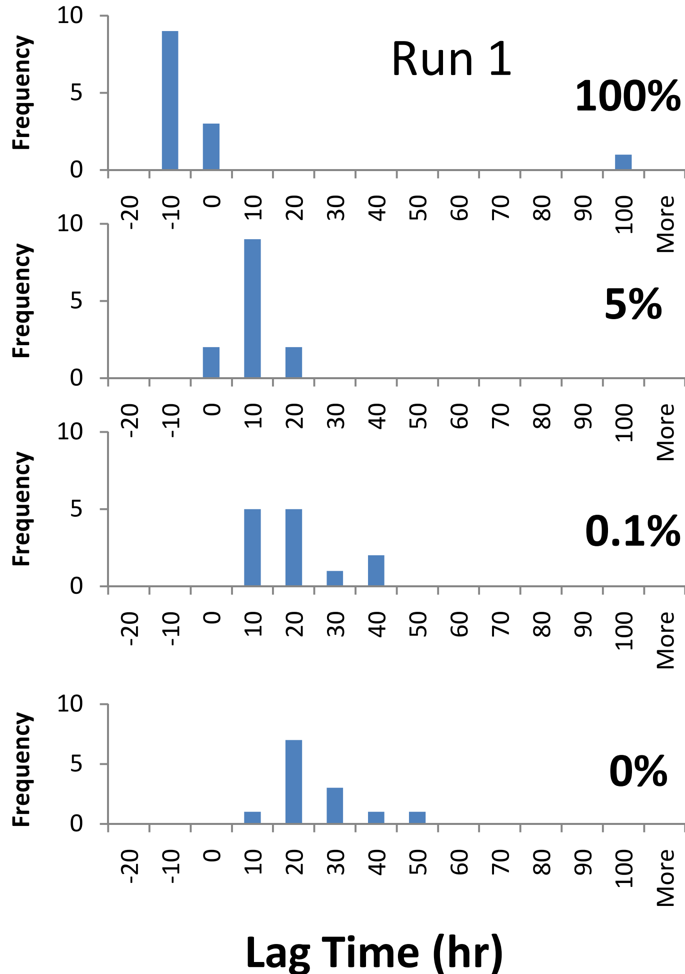


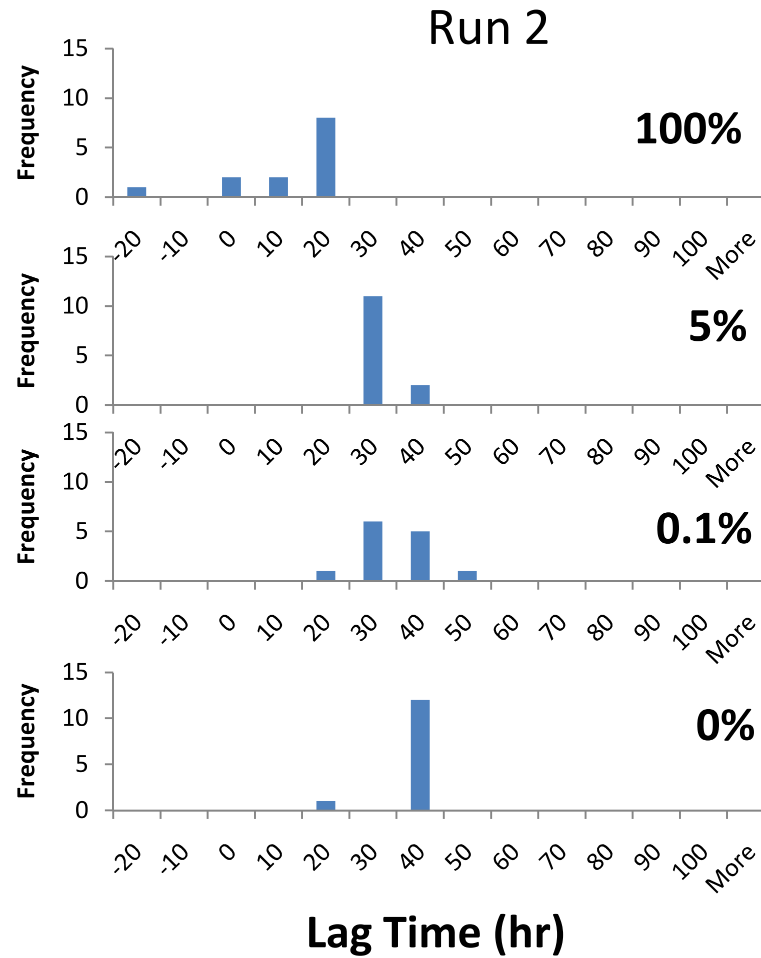
Signaling by Growth factors Transported by Diffusion and Advection? Conditioned Media Experiment



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

$$c_x = \left(\nu \frac{n_0}{\gamma_{slow}}\right)\left(\exp(\gamma_{slow}\, t) - 1\right)$$

Estimate c_x at 6×10^{-10} to 10^{-8} M and K_D as 8×10^{-11} to 2×10^{-8} M typically 5×10^{-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 $\sigma_{t_{lag}}$ of 10 to 15 hours, which implies $\frac{\sigma_{n_x}}{n_x} = \gamma_{slow}\sigma_{t_{lag}}$ = 0.40 to 0.59
- Employ theory of fluctuation (Lauffenburger, 1993) in binding occupancy (θ):

$\sigma_\theta = (c_x K_D)^{\frac{1}{2}}/R^{1/2}(K_D + c)$ 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 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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