Contact-mediated cell-assisted cell proliferation in a model eukaryotic single-cell organism: An explanation for the lag phase in shaken cell culture

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In cell culture, when cells are inoculated into fresh media, there can be a period of slow (or lag phase) growth followed by a transition to exponential growth. This period of slow growth is usually attributed to the cells' adaptation to a new environment. However, we argue that, based on observations of shaken suspension culture of *Dictyostelium discoideum*, a model single-cell eukaryote, this transition is due to a density effect. Attempts to demonstrate the existence of implicit cell signaling via long-range diffusible messengers (i.e., soluble growth factors) through cell-medium separation and microfluidic flow perturbation experiments produced negative results. This, in turn, led to the development of a signaling model based on direct cell-to-cell contacts. Employing a scaling argument for the collision rate due to fluid shear, we reasonably estimate the crossover density for the transition into the exponential phase and fit the observed growth kinetics.

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I. INTRODUCTION

The study of living matter has stressed the central role of intercellular communication in the regulation of tissue growth in normal states (e.g., in embryogenesis) and in disease states (especially cancer) [1]. An elegant opportunity to examine such collective behavior is provided by the reciprocating shaker system used to grow cells in suspension. In contrast to the naturally complicated arrangement of cells on cells or filamentary scaffolding, suspension cultures offer the possibility of communication between freely floating cells. Basic concerns are the degree to which collective behavior is present in such a system, the means by which intercellular signaling is brought about, and the manner in which vital computations in such living matter are conducted [2]. We are particularly interested in a familiar transition in cell population dynamics of a closed culture at low density: the lag-log switchover in which a culture undergoes a dramatic change from slow to exponential growth [3].

While the biology literature [4] highlights the biochemical aspects of cellular communication, the physical basis is often not as well discussed [5]—even though this is clearly vital to tissue formation and the multicellular life of colonies. In this paper, we will endeavor to understand the physical basis of the slow-to-fast growth transition in shaken cell culture of the model eukaryote *Dictyostelium discoideum* and present evidence that it is not due to chemical messengers transported between cells by flowing medium (i.e., endocrine) signaling, but rather contact signaling. Whether or not chemical recognition (i.e., juxtacrine) signaling is required will be left as an intriguing unresolved question, along with the precise magnitude of cooperativity employed in this model example of collective computation in living matter.

The essential question before us is the nature of the well-known, but poorly understood transition in cell culture between slow (lag phase) and exponential (log phase) growth. In the biology literature, the standard explanation of this transition is given from an individual cell perspective. After cells have been transplanted into a new environment, each cell needs time to adapt to its new surroundings and this period is the lag phase [6–8]. A recent review confirms this position, but argues that a greater understanding of the lag phase is needed [9]. While a very broad mathematical discussion has been provided by Baranyi and Roberts [10], the detailed predictions for an explicit model provided in this paper have been hitherto lacking.

The notion that cells need a significant recovery period after being transplanted into fresh culture media seems implausible to us. For better insight into the nature of the lag phase we took a hint from the cell culture literature that a minimum cell density is needed for vigorous growth in eukaryotic cell culture. Paul [11] explains that this can be as low as 20 cells/ml, but is more commonly on the order of 2×10^4 cells/ml for common primary cell lines [12]. In *D. discoideum*, it is also noted that a minimum of about 10^4 cells/ml is needed to establish exponential growth in a standard shaken suspension culture protocol [13].

In summary, the literature of cell culture presents us with interesting phenomena at low cell density: a lag to exponential transition in a closed culture system and a threshold cell density for exponential growth. Our objective is to reveal the nature of these phenomena in a model system.

II. SHAKEN SUSPENSION GROWTH EXPERIMENTS

To clearly observe the transition from lag phase to exponential phase and to test the notion that the lag phase is a recovery effect, we grew several *D. discoideum* shaken suspension cultures of the AX3 line according to a standard protocol: we used stock HL-5 culture media [14] with antibiotics [15] and 150 rpm rotary shaking 25 ml volumes in 6-cm-diameter flasks at 19 °C [16]. Densities above about

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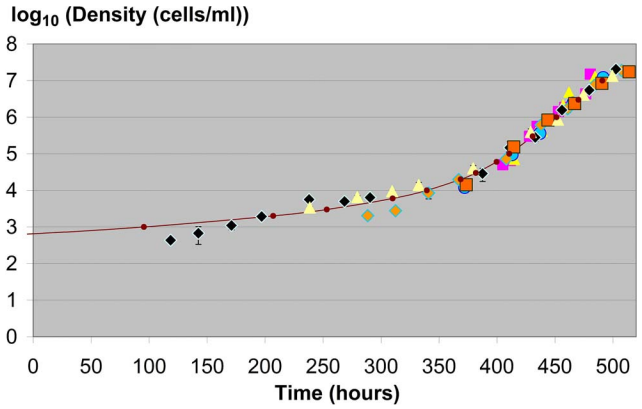

FIG. 1. (Color online) Cell density vs time for shaken suspension cultures. Different symbols indicate different cultures. Start times for low-density cultures are as follows: black diamond run, 0 h; yellow triangle, 185; orange diamond, 170; orange square, 320; and blue circle, 295. The data collapse indicates that the density is the key independent variable. Multiple runs collapsed by shifting time in order to give agreement in the exponential growth phase. A best-fit line to the contact theory with $N_C=1$ and $T_M=0.1$ h is indicated.

2×10^4 cells/ml were measured by direct counting with a hemacytometer [17]. In order to measure lower densities we concentrated cells by a factor of 50 before counting: 1 ml of cell culture was removed (an equal volume of fresh HL-5 culture medium was added to the culture in its place to keep the volume constant [18]), the sample was spun in a 1.5-ml microfuge tube for 30 s [19], supernatant was removed, and cells were resuspended in 20 μl of HL-5 by vortexing for 1–2 min [20]. Where a comparison between direct counts and concentrated cell counts was possible, we saw agreement between the two measurements that was well within a factor of 2. (The sufficiency of this precision can be seen in Fig. 1 in the scatter of the data in successive runs and the fact that the ordinate is a log scale.) The measured growth curves are [21] shown in Fig. 1. The error bars reflect cell counting statistics only. An offset was added to the starting time of each curve to collapse the exponential growth data. The time for each run has been shifted to give the best possible agreement in the exponential growth phase. We see that the agreement is excellent. Most importantly, we see that there is a well-defined lag phase to exponential phase transition that occurs at around 10^4 cells/ml, even though the data consisted of a variety of runs with different initial densities and start times [22]. Runs with starting densities in the exponential regime (not shown so as to minimize pileup in Fig. 1) generally follow the same exponential behavior shown here. The doubling time in the exponential phase is 11.9 ± 0.3 h.

We conclude that for this system the lag to exponential transition is an effect of cell density and not of the time from the start of the culture—i.e., *not* a single-cell recovery effect. This density dependence implies that there must be some counting mechanism employed by the cells, and this in turn implies that the cells must communicate their existence to one another. This communication could occur either (a) via chemical signaling—i.e., a growth factor that accumulates in the medium—or (b) through direct cell-to-cell contacts.

III. EXPONENTIAL PHASE RECONSTITUTION EXPERIMENTS

Let us examine the first possibility—that cells [23] produce a growth factor that is transported between cells in the highly convecting growth medium. Many examples of mobile growth factors are known that return cells to the mitotic cell division cycle from out of the resting *G*0 phase [24]. Recalling the importance of hormone signaling in bloodstreams, we call this possibility endocrine signaling. We first tested for this possibility with exponential growth medium reconstitution experiments by "conditioning" media in an attempt to promote growth as follows. For each experiment, we inoculated AX3 cells into an Erlenmeyer flask containing 30 ml of HL-5. The flask was shaken at 150 rpm. The cells were grown up to exponential phase ($\approx10^6$ cells/ml). We passed 20 ml of this log phase culture though a 0.45-μm syringe filter. We mixed this filtered, cell-free exponential medium with equal parts HL-5. Two flasks (which we denote as *A* and *B*) were each filled with 20 ml of this *reconstituted medium*: 50% fresh, unfiltered HL-5 and 50% filtered exponential phase medium. We then filled two flasks (which we denote as *C* and *D*) with 20 ml of *control medium:* 50% fresh, unfiltered HL-5 and 50% fresh, filtered HL-5 [25]. These four flasks were then inoculated with a small, fixed volume of the original growing cell culture, so that all the bottles contained the same starting cell density, where this density is below the lag—exponential transition density of 10^4 cells/ml. We then measured the density a few days later to see if there was a difference between growth in the reconstituted medium and control medium.

This experiment was conducted four times, and the data are summarized in Table I. The idea behind these experiments is that the low starting cell density should bring the cells in the control flasks back down into lag phase growth, whereas the cells in the reconstituted flasks should remain in exponential growth, because the reconstituted medium should retain the growth factors. Therefore, in order to make useful comparisons between the experiments and the controls, in the last column of Table I, we show the expected final density (EFD) if the cells had been growing exponentially with a 12-h doubling time (which corresponds to the growth rate for the exponential phase in Fig. 1):

$$(\mathrm{EFD}) = (\text{starting density}) \times 2^{(\text{duration})/12\ \mathrm{h}}.$$

If the final density is much lower than the EFD, then the cells have spent considerable time in the lag phase; otherwise, they have not. So in order to find evidence for a growth factor, we need to find cases where the controls have densities well below the EFD and the reconstitution experiments have densities comparable to the EFD.

Experiment 4 does not fit these criteria, because the final densities of the controls are comparable to (they actually exceed) the EFD. This indicates that the controls are growing exponentially already, and so we should not expect to see any improvement in the reconstituted medium. In fact, the densities between the controls and the experiments are quite comparable.

TABLE I. Results of exponential growth medium reconstitution experiments. EFD refers to the expected final density for exponential growth.

Expt	Reconstitution?	Starting Density (cells/ml)	Final Density (cells/ml)	Duration (days)	EFD (cells/ml)
1A	Yes	7.0×10^3	7.0×10^5	5	7.2×10^6
1B	Yes	7.0×10^3	1.0×10^6	5	7.2×10^6
1C	No	7.0×10^3	7.8×10^5	5	7.2×10^6
1D	No	7.0×10^3	6.6×10^5	5	7.2×10^6
2A	Yes	2.0×10^3	3.3×10^6	8	1.3×10^8
2B	Yes	2.0×10^3	1.3×10^6	8	1.3×10^8
2C	No	2.0×10^3	1.8×10^4	8	1.3×10^8
2D	No	2.0×10^3	4.0×10^4	8	1.3×10^8
3A	Yes	4.0×10^3	1.2×10^6	5	4.1×10^6
3B	Yes	4.0×10^3	1.3×10^6	5	4.1×10^6
3C	No	4.0×10^3	1.4×10^6	5	4.1×10^6
3D	No	4.0×10^3	1.1×10^6	5	4.1×10^6
4A	Yes	2.1×10^3	3.5×10^5	3	1.3×10^5
4B	Yes	2.1×10^3	1.0×10^5	3	1.3×10^5
4C	No	2.1×10^3	2.6×10^5	3	1.3×10^5
4D	No	2.1×10^3	1.8×10^5	3	1.3×10^5

Experiments 1, 2, and 3 are potentially good candidates for seeing an effect, because the average final control densities are (respectively, for each experiment) a factor of 10, 4500, and 3.3 lower than the corresponding EFD. Therefore, in these controls, the cells have been growing in lag phase. The ratios between the average final reconstitution and average final control density in these three experiments are 1.2, 79, and 1.0. In the longer-duration experiment 2, we observe a reconstitution density to control density ratio which would be consistent with the action of a growth factor in the medium; however, as the final density in the reconstituted cultures is still much less than the expected final density, we can conclude that in this experiment, as in experiments 1 and 3, the reconstituted medium was not sufficient to restore the cultures to exponential phase growth after a reduction in density. Collectively, these results indicate that growth factors in the medium do not explain the observed density-dependent growth rates.

IV. FLOW-PERTURBED MICROCHAMBER GROWTH EXPERIMENTS

If endocrine signaling were responsible for the observed cell-assisted cell growth, then a sufficiently fast fluid flow past a population of cells should slow their division by carrying away growth factors. To check for this effect, we compared the growth rates of cells in the PDMS on glass [26] flow chamber (glass on bottom, square footprint 2 mm on a side, 200 μm tall) shown in Fig. 2, under a range of fluid flow conditions. Cells taken from shaken culture were injected (typically 100 μl in a few seconds) through the outlet channel (during which time, fresh HL-5 was introduced through the inlet at about 50 μl per minute, with the two unused ports open to avoid introducing cells into the inlet channel [27], which could lead to the growth factor produced in the inlet being swept into the main chamber and stimulating growth in an uncontrolled manner). Following injection, the inlet flow rate of fresh HL-5 media was quickly reduced to the particular value to be explored. After a few minutes, cells adhered to the glass bottom of the microchambers and were not dislodged by the steady flow of culture medium passing diagonally across the chamber (the side ports having been closed). Rates of controlled volumetric injection of medium were chosen to explore a range of expected degrees of perturbation of endocrine signaling, as estimated by the ratio of advective to diffusive transport in the microchamber. This ratio is measured by the Péclet number [28] $\mathrm{Pe}\equiv uL/D$, where u is the characteristic flow speed, L a characteristic signaling length, and D the diffusion constant of the hypothetical signaling molecule. Assuming that any signaling molecule will be light weight and therefore have diffusivity

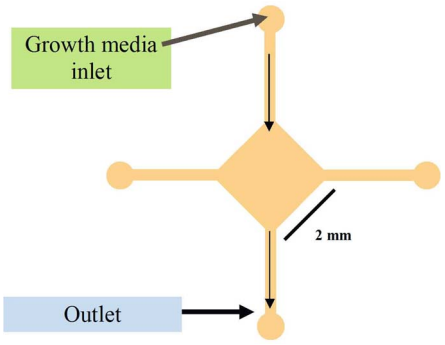

FIG. 2. (Color online) Schematic of microchamber used for flowing medium growth experiments. See text for details.

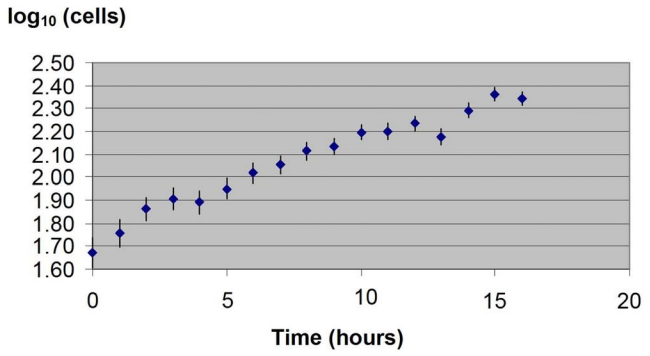

FIG. 3. (Color online) Cell count in microchamber (less fixed background) vs time. Media flowing with Pe approximately 1.

on the order [29] of 300 $\mu m^2/s$, estimating the characteristic length as 100 μm (a reasonable fraction of the microchamber's lateral dimensions), and taking the channel width in the device to be the diagonal length 1.4 mm, we find that a volume flow rate of roughly 0.04 μl per minute provides Pe=1. For other Peclet values, we adjusted the volume flow rate accordingly.

Images were taken every 15 min in bright field through a digital microscope [30]. Using the ImageJ [31] image processing programming environment, frames were Fourier filtered, segmented into cell-sized objects, and automatically counted after intensity thresholding. A fixed pattern background count derived by averaging an early five frames in the time series was subtracted. A typical growth curve derived in this manner is shown in Fig. 3.

Using cell counts on the order of 100 per chamber, we found exponential growth with the doubling times as a function of Pe as given in Table II. The doubling time is close to, but perhaps a bit shorter than, that seen in the exponential phase of shaker culture growth.

We see no sign that log phase growth is disrupted or attenuated by fluid motion in this important range of flow speeds [32]. In conclusion, in searching for the means by which cells communicate their presence to each other we must consider an alternative to endocrine signaling.

V. CELL-ASSISTED CELL GROWTH VIA DIRECT CONTACTS

Rather, we considered the possibility of contact signaling. We found strong evidence for such interactions by turning to direct observation of exponential phase cultures. There, we noticed that clumps of cells could be found in high-density suspensions. A sample spotted out shows such a clump in

TABLE II. Growth doubling time in exponential phase vs Péclet number (ratio of transport rate for convection over diffusion).

Pe	Doubling time (hours)
0.4	9.9 ± 1.3
1.0	8.0 ± 1.0
10.0	8.9 ± 1.4

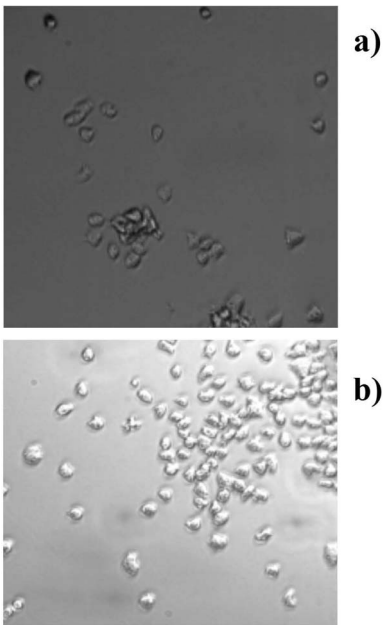

FIG. 4. Sample of shaken suspension culture (a) immediately after sample was taken indicating clumps (b) 2.5 h later at the same location, indicating instability of clumps. Individual cells are about 14 μm across.

Fig. 4(a). Figure 4(b) shows that they are in fact unstable on glass substrates such as one finds in the microchambers, suggesting why they were not noticed in that work. The contact signaling notion provided us with a possible means of understanding cell-assisted cell growth [33]. One can argue as follows: as in many computations of life, a switchlike mechanism is in order: if a cell at low density makes a sufficient number of contacts (N_C) with other cells in a period of time (T_M) that corresponds to the cellular measuring interval, then we can argue that the cell will be cleared for division at a rate (γ) that corresponds to familiar exponential growth at high densities. To summarize, we assert that cells sense growth-stimulating density by means of collisions. In order to quantify this effect, we will estimate the mean time between collisions (τ) and use Poisson statistics to find the fraction of the population (P_G) that meets the criterion to be switched for growth by collisions. Our dynamical equation for the cell density (n) is

$$\dot{n} = \gamma P_G n. \tag{1}$$

We argue for P_G's dependence on n as follows [34]. First, we find the dependence of τ by means of scaling arguments: we expect that the shaken media cause cells to move in a well-mixed diffusive manner. Hence we expect τ to vary with the mean separation between cells, $n^{-1/3}$, as

$$n^{-1/3} \propto \tau^{1/2}. \tag{2}$$

Just as cars in different lanes of a unidirectional highway catch up with each other at a rate that is proportional to their relative speed which depends on their separation across lanes, we also expect τ to depend on the average fluid shear rate [35] S (whose dimensions are inverse time) as follows:

$$\tau \propto S^{-1}. \tag{3}$$

Combining Eqs. (2) and (3),

$$\tau \propto n^{-2/3} S^{-1}. \tag{4}$$

We use the size of a cell (a) to restore units:

$$\tau \approx a^{-2} n^{-2/3} S^{-1}. \tag{5}$$

Note that as expected the mean time between collisions drops as the cross section for contact grows with cell size.

We can now estimate S by dimensional analysis by considering the size of the diameter of the shaker flask (L) and the period of the shaker (T). From this we form a characteristic fluid speed L/T and use this to estimate the shear rate (a velocity gradient):

$$S \approx (L/T)/L = T^{-1}. \tag{6}$$

Applying this result to Eq. (5), we complete our scaling estimation for τ:

$$\tau \approx a^{-2} n^{-2/3} T. \tag{7}$$

We can now apply Poisson statistics to find P_G as follows. The average number of collisions (λ) during the measurement time is given by

$$\lambda = T_M/\tau. \tag{8}$$

Given that the Poisson probability of having k collisions in time interval T_M is [36]

$$p(k,\lambda) = \exp(-\lambda)\lambda^k/k\,!\,, \tag{9}$$

we can find the probability of switching to growth with N_C or more collisions in the measuring interval from

$$P_G = p(N_C,\lambda) + p(N_C+1,\lambda) + p(N_C+2,\lambda) + \cdots.$$

This is readily summed via Eq. (9) to give

$$P_G = 1 - \exp(-\lambda) \sum_{k=0}^{N_C-1} \lambda^k/k!. \tag{10}$$

We now proceed to find growth time (t) as a function of cell density by integrating Eq. (1) with P_G's dependence on n given by replacing λ with the following from Eqs. (7) and (8):

$$\lambda = a^2 n^{2/3} (T_M/T). \tag{11}$$

Examining Eq. (10) reveals the crossover behavior of P_G as a function of n: from vanishing probability of growth at low density to complete assurance of growth at high density, with an increasingly switchlike behavior as cooperativity grows with increasing N_C. Numerically integrating [37] down from the largest values of n (to avoid small differences between large numbers) we find the desired time intervals (Δt's) using

$$\int_{n=n_1}^{n_2} \frac{dn}{nP_G(n)} = \gamma(t_2 - t_1) = \gamma\,\Delta t. \tag{12}$$

From Eq. (12) we see that γ^{-1} sets the time scale for growth dynamics. We can now see how Eq. (12) compares with the observations of Fig. 1. The shake period (T) was fixed by the inverse of the shake rate employed, 150 rpm. The cell size (a) was taken to be 10 μm, based on observation. Although we expect the measurement time T_M to be well below the cell cycle time (given by γ^{-1}, which is on the order of 10 h from the exponential phase data), we also anticipate that the memory of at least one previous cell collision might involve nuclear regulation and control of the cell cycle. King *et al.* [38] recently reported gene activation and expression experiments which suggest that T_M is at most hours long. Traverse *et al.* [39] find that gene activation by an extracellular growth factor can occur over time scales as rapid as several minutes. We therefore estimate a time scale for T_M on the order of 0.1–1 h.

Following the discussion after Eq. (11), one can estimate by inspection that the lag to exponential crossover will occur at $\lambda \approx 1$. Substituting into Eq. (11) with our estimated range in T_M yields a crossover density on the order of $10^3 - 10^4$ cells/ml, consistent with the crossover density seen in Fig. 1. In order to complete the comparison of the contact theory given by Eq. (12) with the growth curves of Fig. 1, we surveyed various values of the parameters N_C and T_M as follows: using a wide series of density values we first adjusted γ and a time offset (an arbitrary quantity in the experiment) to match the exponential phase [40]. This gave $\gamma^{-1} = 17.1 \pm 0.5$ h. We also adjusted T_M for each value of N_C explored. We found that a wide range of N_C starting with the smallest possible value 1, described our data. By increasing N_C to as large as 12 we get a hint that this value is larger than indicated by the data since the theory begins to exhibit too sharp a crossover. At the high value of $N_C = 12$, we found that $1.5 < T_M < 6$ h. For the lowest possible value of N_C—namely, 1—we found that $0.08 < T_M < 0.2$ h gave a good account of the data. A typical fit is shown in Fig. 1 for $N_C = 1$ and $T_M = 0.1$ h. The dispersion of the measurements at low densities gives the dominant uncertainty.

Note that in the course of our measurements we did not examine such a low density as to indicate the necessity of including within the theory a spontaneous growth term. We expect such a possibility, since it has been reported that *D. discoideum* colonies can be routinely grown from a single cell [41]. While we find that a broad range of cooperativity (as expressed by the parameter N_C) is consistent with our data, the most biologically efficient possibility $N_C = 1$ is completely plausible, having a corresponding biocomputation processing time that is consistent with our expectations.

VI. CONCLUSION

To summarize, we assert that, contrary to popular descriptions, the slow-to-exponential growth transition in the shaken culture of the model eukaryote *D. discoideum* is not a sign of recovery of a lone cell from entrance into a new environment, but rather an example of multicellular behavior [42]. We have shown that the signaling that alerts cells to each other's presence is accomplished not by means of media-transported growth signal factors. Rather, we argue that signaling occurs by means of direct cell contact, which could be expected to rely on recognition through membrane bound ligands and receptors. We have shown that a plausible and

efficient computation scheme for this organism to discover whether an individual cell has received a sufficient number of contacts to execute the mitotic cycle is consistent with the measured growth curves for shaken suspension culture. The possible universality of such a mechanism is supported by the recent view by Ingber of such a mechanochemical programming process as a crucial biological phase transition [43]. A vital consideration is the evolutionary advantage that this cell assisted cell growth might reward this class of organisms. One thought is that the presence of a modest number of neighboring cells might indicate to each cell a propitious environment: perhaps one rich in nutrients (as opposed to the inhibition of growth that might occur in crowded, stationary phase, circumstances [44]). A cell might need this assurance of metabolic supply before making the resource commitment for cell division. Another idea is that since sexual reproduction provides a powerful evolutionary chance at survival of extreme conditions, a variety of individuals are needed for crosses, hence the advantage of establishing a colony with more than just the clones of a single progenitor cell [45].

Very recently we have obtained preliminary evidence that one of our twice-frozen AX3 cell lines is in fact a new strain (in our laboratory) lacking a lag phase. This is indeed a fortuitous development since it is obvious that such a phenotype would be favored over the usual lagging strain by our standard, low-stress, culture protocol. One immediately sees that the existence of such a strain suggests that the lag-exponential apparatus represents a control system overlaid on a more primitive system which lacks a density triggered means of arresting the cell division cycle (we had in fact planned a mutation effort to knock out the control system).

In follow-up work it would be valuable to uncover the biochemical details of the contact signaling and memory mechanism for which we have argued. It would be important to examine shaken cultures for other organisms and discover the degree of specificity of contact signaling with mixed cultures. Also, to what extent is living matter required to stimulate growth through contact signaling? Are mechanical contacts sufficient to bring a cell from out of the $G0$ phase? Is there, in fact, a lag phase in *D. discoideum* cell culture on substrates? That is to say, are such surfaces made favorable for cell division simply by washing them with HL-5 growth media, which is rich in proteins and carbohydrates [46]? The idea that cells are brought to the exponential growth phase by contact with a generic substrate is consistent with our microchamber results. In fact, the standard procedure [47] for growing *D. discoideum* cells from frozen stock with a low concentration of viable cells is to first culture on a substrate, very likely the approach employed to grow a colony from a single cell as mentioned earlier. In a general discussion of nontransformed (i.e., noncancerlike) tissue culture, it is pointed out that "most cells need to attach to the extracellular matrix to … proliferate." [48] Thus a vital follow-up to the present work is to explore exceedingly low-density growth on various (adhesive and nonadhesive) surfaces with particular attention paid to spatial patterning on the substrate surfaces and in the resultant colonies.

At the suggestion of a referee, we consider the possibility that the signaling is not due to direct contacts, but rather through growth factor(s) in solution that are degraded before diffusing very far (an example of the paracrine form of signaling mentioned in the references). This notion is readily incorporated into our model by simply increasing the effective cross section for cell-cell collisions. The flow perturbed growth experiments would have to be extended to significantly greater flow rates [49] in order to test this possibility. It certainly suggests the manner in which cells in clusters might be signaling each other. At this time, we cannot rule out the possibility that paracrine signaling can be sufficiently short ranged so as to be indistinguishable from juxtacrine signaling. Turning to another theoretical front, it would be useful to reexamine the fluctuation-enhanced mean-field theory for growth that we have proposed with more sophisticated approaches that capture fluctuations effects more explicitly possibly through a more numerical effort than the bare-bones analytical theory given here. Clearly the alteration by experiment and improved modeling (particularly for colloidal collisions under mixing flow) of the hydrodynamic environment of shaken and stirred suspension cultures should be useful. An important step is to examine the effect of varied stir rates on a spinning impeller culture system, for which our contact signaling model makes a clear prediction of a supralinear shift in the lag-exponential crossover density as a function of stir rate. Genetic effort including sequencing aimed at interesting circuits would be an appropriate major follow-up to our investigation of a lagless strain. Finally, the further study of what promotes cell growth among a few cells is as important for the biotechnology of massively parallel cell culture as it is for food contamination, tissue generation, embryogenesis, and cancer formation.

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[1] Gerhard Krauss, *Biochemistry of Signal Transduction and Regulation*, 3rd ed. (Wiley-VCH, Weinheim, 2003), p. 429.

[2] The problem of collective computation and communication in synthetic systems is also of great interest in contemporary wireless technology; see a description of networks of autonomous sensors, possibly mobile, that mimic cell colonies by Edgar H. Callaway, *Wireless Sensor Networks: Architectures and Protocols* (Auerbach Publications, Boca Raton, 2004) and a discussion of cognitive radio networking by Steven Ashley, Sci. Am. **293** (3), 66 (2006).

[3] Note that we are not discussing the highest density or "stationary" phase where limited metabolic resources and the buildup of waste products create complicated situations.

[4] *Handbook of Cell Signaling*, edited by Ralph A. Bradshaw and Edward A. Dennis (Academic, Amsterdam, 2004).

[5] If only to be judged by the number of times the word biochemistry appears as a qualifier in the titles of texts on cell signaling. In any case the physical aspects of cells signaling discussions while very descriptive structurally, usual lack quantitatively falsifiable theory.

[6] J. G. Black, *Microbiology*, 4th ed. (Prentice-Hall, Upper Saddle River, NJ, 1999), pp. 138–140.

[7] L. M. Prescott, J. P. Hurley, and D. A. Klein, *Microbiology*, 6th ed. (McGraw Hill, Dubuque, 2005), pp. 110–112.

[8] S. John Pirt, *Principles of Microbe and Cell Cultivation* (Blackwell, London, 1975) p.195.

[9] I. A. M. Swinnen, K. Bernaerts, E. J. J. Dens, A. H. Geeraerd, and J. F. Van Impe, Int. J. Food Microbiol. **94**, 137 (2004).

[10] Jozsef Baranyi and Terry A. Roberts, Int. J. Food Microbiol. **26**, 199 (1995).

[11] John Paul, *Cell and Tissue Culture*, 5th ed. (Churchill Livingstone, Edinburgh, 1975), p. 34.

[12] Cells taken from a living organism.

[13] http://dictybase.org/techniques/media/general_dicty_techniques.html#growth

[14] 25 ml of aqueous Formedium HL-5 with glucose, catalog No. HLG0102 ForMedium Ltd., Norwich, UK.

[15] 0.25 ml of Gibco Catalog No. 15140–122 penicillin and streptomycin.

[16] http://dictybase.org/techniques/media/general_dicty_techniques.html#growth using a Lab-Line Model 4626 Barnstead/Thermolyne shaker.

[17] A chamber used to count cells under a microscope using a 10 μl sample. See http://dictybase.org/techniques/media/general_dicty_techniques.html#growth We used a Bright-Line model hemacytometer chamber from Hausser Scientific with a coverslip.

[18] We found that the effect of successive dilutions due to the 1 ml out of 25 ml sampling gave an insignificant correction. In the most sensitive portions of the data (the most samples, lowest concentration: the black diamonds in Fig. 1 for densities below 10^4 cells/ml) we estimate that successive dilutions lowers the concentration at 10^4 cells/ml, the lag to exponential crossover by 9%.

[19] National Labnet type C-1200 minicentrifuge.

[20] Using a Barnstead/Thermolyne Model M633215 vortexer.

[21] For initial densities below about 10^4 cells/ml.

[22] Attempts were made to deduce initial densities by dilution from the source cultures, but our procedures, now adequate to the task, were not sufficiently accurate to provide credible results at that time. Therefore the only very low density results reported here were according to the concentration method described.

[23] S. John Pirt, *Principles of Microbe and Cell Cultivation* (Blackwell, London, 1975), p. 198.

[24] Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter, *Molecular Biology of The Cell* (Garland Science, New York, 2002), pp. 871, 1015, and 1019.

[25] Note that with the dilution factor of 2 for the conditioned medium, we expect, assuming that a growth factor is present, to have a growth factor concentration corresponding to a cell density of 5×10^5 cells/ml, well within the exponential regime.

[26] Following David C. Duffy, J. Cooper McDonald, Olivier J. A. Schueller, and George M. Whitesides, Anal. Chem. **70**, 4974 (1998).

[27] The inlet rate used was found empirically to prevent cells from being injected into the inlet channel.

[28] Ronald F. Probstein, *Physicochemical Hydrodynamics*, 2nd ed. (Wiley-Interscience, New York, 1994), p.48.

[29] Kensal Edward Van Holde, *Physical Biochemistry* (Prentice-Hall, Englewood Cliffs, NJ, 1971), p. 89.

[30] Olympus IX71 microscope with a 2.5× objective and Media Cybernetics Evolution QEi camera.

[31] http://rsb.info.nih.gov/ij/

[32] We note that in estimating the significance of the flow, we assumed that the putative growth factor was a small molecule. This is a conservative estimate since larger molecules would have a lower diffusivity and hence lead to even more dramatic flow perturbation (i.e., a larger Péclet number) than what we estimated.

[33] Richard O. Hynes, Cell **69**, 11 (1992), suggests that contact signaling by a variety of cell surface proteins called integrins might well mimic growth signals transmitted by intercellular soluble growth factors. Ernst J. M. Helmreich, *The Biochemistry of Cell Signalling* (Oxford University Press, Oxford, 2001), p. 66, also points out that integrins can be regulated by chemical contact and intriguingly for our present discussion can promote cell clustering. He notes (p. 7) that signaling ligands (including growth factor ligands) can serve in both membrane bound and diffusible forms. While integrins might well be expected to respond to nonspecific targets, Joan Massague and Atanasio Pandiella, Annu. Rev. Biochem. **62**, 515 (1993), argue that membrane-anchored growth factors might enable highly selective signaling (and that the same biochemical moiety might serve as both ligand and receptor). She calls such contact signaling juxtacrine signaling in order to contrast it with autocrine (same cell signaling via diffusion through extracellular medium), paracrine (between nearby cells through diffusion through the extracellular medium) and endocrine signaling which all require diffusible growth factors.

[34] In developing a description of the lag-exponential transition at low cell density, one is first tempted to perform an expansion in density and therefore begin with the following alternative to Eq. (1): $\dot{n}=\gamma n+\kappa n^2+\cdots$, where γ and κ are constants. [We interpret the interaction term $(\kappa n)n$ with $\kappa>0$ as indicative of a density-dependent growth rate favorable to proliferation.] Such an approach utterly fails: departures from simple exponential growth occur with increasing not decreasing density as observed in Fig. 1.

[35] Ronald F. Probstein, *Physicochemical Hydrodynamics*, 2nd ed. (Wiley-Interscience, New York, 1994), p. 249.
[36] http://mathworld.wolfram.com/PoissonDistribution.html
[37] Using MATLAB 7.0 by Mathworks.
[38] Kevin R. King, Sihong Wang, Daniel Irimia, Arul Jayaraman, Mehmet Toner, and Martin L. Yarmush, Lab Chip **7**, 77 (2007).
[39] Sarah Traverse, Nestor Gomez, Hugh Paterson, Chris Marshall, and Philip Cohen, Biochem. J. **288**, 351 (1992).
[40] Using Microsoft Excel 2003.
[41] David Knecht (private communication).
[42] See, for example, quorum sensing studies in bacterial colonies as described in A. Camilli and B. L. Bassler, Science **311**, 1113 (2006).
[43] Donald E. Ingber, J. Cell. Sci. **116**, 1397 (2003).
[44] D. A. Brock and R. H. Gomer, Development **132**, 4553 (2005).
[45] Social amoeba employ genetic recombination under harsh circumstances as detailed in Richard H. Kessin, *Dictyostelium: Evolution, cell biology, and the development of multicellularity* (Cambridge University Press, New York, 2001), p. 22, and the work of Day—e.g., Keith E. Lewis and Danton H. Day, Nature (London) **268**, 730 (1977). We are arguing that diversity in vegetative colonies such as we are studying could be an essential feature for sexual reproduction to have evolutionary value for the species.
[46] www.formedium.com/uk/DictyosteliumDiscoideum/DictyosteliumDiscoideum.htm
[47] http://dictybase.org/techniques/media/general_dicty_techniques.html#growth
[48] Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter, *Molecular Biology of the Cell* (Garland Science, New York, 2002), p. 1110.
[49] To give a significant Peclet number since compared to our microchamber experiments the characteristic distance is reduced to the spacing between nearby cells as opposed to a good fraction of the chamber's larger dimensions.