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Mini Review

The Use of Diffusion Calculations and Monte Carlo Simulations to Understand the Behavior of Cells in *Dictyostelium* Communities

Richard H. Gomer *

Department of Biology, Texas A&M University, College Station, TX 77843-3474, USA

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ABSTRACT

Microbial communities are the simplest possible model of multicellular tissues, allowing studies of cell-cell interactions to be done with as few extraneous factors as possible. For instance, the eukaryotic microbe *Dictyostelium discoideum* proliferates as single cells, and when starved, the cells aggregate together and form structures of ~20,000 cells. The cells use a variety of signals to direct their movement, inform each other of their local cell density and whether they are starving, and organize themselves into groups of ~20,000 cells. Mathematical models and computational approaches have been a key check on, and guide of, the experimental work. In this mini-review, I will discuss diffusion calculations and Monte Carlo simulations that were used for *Dictyostelium* studies that offer general paradigms for several aspects of cell-cell communication. For instance, computational work showed that diffusible secreted cell-density sensing (quorum) factors can diffuse away so quickly from a single cell that the local concentration will not build up to incorrectly cause the cell to sense that it is in the presence of a high density of other cells secreting that signal. In another example, computation correctly predicted a mechanism that allows a group of cells to break up into subgroups. These are thus some examples of the power and necessity of computational work in biology.

Contents

1. Introduction

Microbial communities exit in environments ranging from deep sea vents to soil to the surfaces and interiors of plants and animals. In some of these communities, the cells can communicate with each other, typically by releasing or secreting factors that other cells can sense. For

* Corresponding author at: Department of Biology, Texas A&M University, ILSB 301 Old Main Drive, College Station, TX 77843-3474, USA.
E-mail address: rgomer@tamu.edu.

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instance, in communities of the eukaryote *Dictyostelium discoideum*, cells can sense the local density of other *Dictyostelium* cells, sense the location of the center of the community, sense the local density of starving cells, and sense attractive signals that guide cells to form multicellular aggregates. For all of these processes, computational approaches have played a key role in our understanding of these remarkable aspects of the behavior of a microbial community.

Dictyostelium cells are small eukaryotic cells which live on soil surfaces and phagocytose and digest nutrients such as bacteria and other microorganisms [1]. The amoebae are motile, and while moving to find food (the cells can sense and move towards individual bacteria), the cells tend to disperse. As the cells proliferate, the community expands, and eventually the cells overgrow the available nutrients and starve. The starved cells then aggregate using relayed pulses of extracellular cyclic adenosine monophosphate (cAMP) as a chemoattractant, and form multicellular aggregates that then form 1–2 mm tall fruiting bodies consisting of a mass of spore cells held up by a thin column of stalk cells. The spores are dispersed by the wind, and if the spore lands in a moist environment, it will become an amoeba that can start a new community of cells. *Dictyostelium* is a premier system for studying secreted signals and the physics of development for several reasons. The first is the simplicity of cells differentiating into just two main cell types and forming structures that can be seen with the naked eye. Second, there are a wide variety of genetic tools [2–7], mutations that completely block development often do not inhibit proliferation, and mutants can be stored frozen. Third, cells grow as plaques on lawns of bacteria on agar plates, allowing easy visual screening for developmental mutations. Finally, the cells grow at room temperature, allowing easy microscopy of live cells, and grow in an inexpensive serum-free defined medium, facilitating purification of secreted factors.

2. Results

2.1. Theoretical and Computational Work Was and Is an Integral Part of Understanding Dictyostelium Aggregation

Some of the earliest computational/ theoretical work to understand the behavior of cells in a microbial community was used to model how starved *Dictyostelium* cells aggregate [8–20]. In a field of starved *Dictyostelium* cells, some cells will begin secreting pulses of cAMP. Nearby cells (a second cohort) will sense the cAMP, and simultaneously secrete a pulse of cAMP and move towards the source of the first cAMP pulse. Cells further away from the source of the original cAMP pulse, but near the second cohort, sense the cAMP from the second cohort, relay the cAMP pulse to cells even further away, and move towards the second cohort. The pulses repeat and spread through the field every ~6 min, and to avoid extracellular cAMP concentrations building up and swamping the cAMP receptors on cells, the cells secrete a cAMP-degrading enzyme. With this mechanism, 10 μm diameter cells over a ~1 cm diameter field can aggregate together. Computational work has guided and checked all aspects of the studies on this mechanism, from the extracellular signal concentrations, to the receptor interactions, down to detailed models of how a slight gradient of cAMP sensed by cells activates specific proteins in the signal transduction mechanism which regulate specific proteins in the cytoskeleton to direct cell movement towards the source of the pulse of cAMP [8–20]. Computational approaches have even successfully modeled the morphogenesis of the aggregated cells into structures that are about to form fruiting bodies [21]. Because the vast scope of this computational work is beyond what could fairly be addressed in a minireview, this will not be addressed in this minireview.

2.2. Computational Approaches to Understanding how Cells Sense the Number or Density of Other Cells

A longstanding idea in developmental biology is that an organism could regulate the size of a tissue containing type X cells, or regulate the number of type X cells throughout the organism, if the type X cells secrete a characteristic diffusible factor x, simultaneously sense the concentration of x, and slow or stop proliferating when the concentration of x reaches a threshold. Such factors are called chalones, and there is good evidence that these regulate the size of tissues such as the spleen in mammals [22–25]. Unfortunately, most chalones have eluded identification, and we have been using *Dictyostelium* to purify and elucidate chalones. A key test for a chalone is that at equilibrium in the tissue or body, the type X cells should be secreting the chalone at a rate sufficient to reach the steady-state concentration of the chalone where it inhibits type X cell proliferation. In a body with an available extracellular volume V, with a number of X cells N_X secreting x at a rate of ϕ molecules per minute, and x having an average lifetime of τ minutes, the steady state x concentration is simply $[x] = N_X\phi\tau/V$. Biochemical purification of x from cells and assessment of the x lifetime can then be compared to measurements of the effect of different concentrations of x on cell proliferation to check that x is indeed acting as a chalone.

Because mammalian chalones have eluded identification, we have used the advantages of *Dictyostelium* to identify a variety of factors that *Dictyostelium* cells use to inhibit their own proliferation, as well as to sense the composition of a *Dictyostelium* community. However, *Dictyostelium* cells grow as communities on surfaces that tend to be much larger than the size of the colony of cells, such as the surface of soil or the surface of a leaf, typically starting from a single spore, so rather than there being a simple confined environment as in a body, the geometry is typically a disk of cells on a relatively large surface, and this makes the signal concentration calculations needed to check that the signal is acting in the hypothesized manner considerably harder. For a cell on a surface that momentarily secretes a signal x, the concentration of x will be a steadily widening Gaussian distribution centered on the location of the cell. For a cell continuously secreting x, the concentration will thus be an integral over time of a series of Gaussian distributions; narrow distributions at short times, and wide distributions at longer times. These integrals are related to error functions, and cannot be solved in closed form. However, they can be converted to infinite series such as

$$[x] = \frac{-\phi}{4\pi Dh}\left[0.5772156649 + \ln\left(\frac{r^2}{4Dt}\right) + \sum_{n=1}^{\infty}\frac{\left(-r^2/4Dt\right)^n}{n!n}\right]$$

for cells in a thin layer of liquid of height h on an impermeable surface such as a leaf, and

$$[x] = \frac{\phi}{2\pi^{3/2}Dr}\left[\sqrt{\pi} - \sum_{n=0}^{\infty}\frac{(-1)^n\left(\frac{r^2}{4Dt}\right)^{(n+1/2)}}{n!(n+1/2)}\right]$$

for cells on a thick layer of a permeable material such as dirt or agar [26]. In the above equations, [x] is the concentration of x at a distance r from the cell at a time t after the cell started secreting x at a rate of ϕ molecules per minute, and D is the diffusion coefficient of x. This can easily be solved with a few lines of code, and to calculate [x] at a point A in or near a community of cells (Fig. 1), a few more lines of code can sum the contribution from every cell in the community to the local concentration of x at point A [26,27]. A simple albeit somewhat messy correction can then be made to adjust for the presence of receptors for x on cells, which by binding x reduce the concentration of x [26]. Because the amount of x bound to a cell is a function of the number of receptors per cell, the K_D of the receptor for x, and the local concentration of x, this needs to be calculated for each cell in the community to generate a new distribution of x concentrations. This then changes the x concentrations needed to calculate the amount bound to cells, so the procedure needs to be done iteratively. Using these computational approaches, we showed that a factor secreted by *Dictyostelium* cells indeed acts as a cell density sensing factor, activating cells when there are physiological

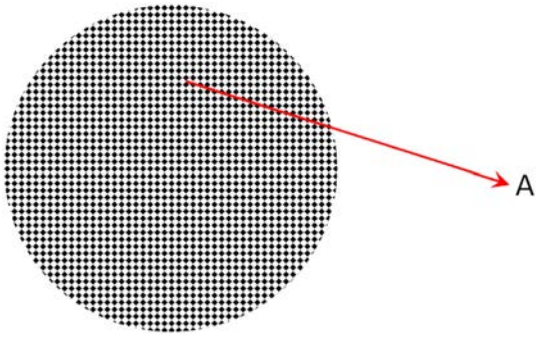

Fig. 1. Calculating the concentration of a secreted factor. For the cell (diamond shape) at the beginning of the red arrow in a disk-shaped community of cells, computation to sum an infinite series until the terms become arbitrarily small gives the concentration of the factor at the point A at a distance r from the cell at some time after the cells have begun secreting the factor. Subsequent computation of the contribution of all other cells in the community, and summation of these contributions, gives the total concentration of the factor at point A. The computation can then be repeated for a series of points starting at the center of the community and moving radially outwards to give the concentration profile of the factor across and beyond the community. Iterative corrections then can adjust for receptors on cells decreasing the free concentration of the factor.

numbers and surface densities of cells in a community, and not activating the cells at low densities or low total numbers where cells had been observed to not begin the developmental step that we thought the factor mediated [26]. Note, however, that this computation does not take into account the lifetime of the secreted factor. A finite lifetime of the factor would tend to decrease the concentration of the factor at each point. A testable prediction is that because molecules of the factor far from a cell would tend to be older than molecules of the factor close to the cell, a finite lifetime of the factor would tend to decrease factor concentrations (on a percent basis) far from a cell more than the percent decrease close to the cell. Other parameters that could be added to this model are secretion and breakdown rates of the factor that depend on the factor concentration or the concentration of a different factor, and boundary conditions instead of the infinite 2- or 3- dimensional manifold [28,29].

In another instance, we had found that a secreted factor acts as a chemorepellent, allowing cells at the edge of a community of cells to sense the gradient between the high concentrations of the factor in the community and the low concentration outside the community, and use this gradient to move away from the community to find new sources of food [30]. A recombinant version of the factor also acted as a chemorepellent in gradient chambers [30], but to make sure we were using biologically relevant gradients, we did concentration calculations as described above to determine the physiological concentration range and slope (d[x]/dr), where r is the distance moving radially outwards from the colony (Fig. 2A) [27].

2.3. Computation Solves the Problem of a Cell Tricking itself

A puzzling aspect of the idea that a secreted factor can allow cells to sense their local density was that one can envision a solitary cell continuously secreting a cell-density sensing factor, and the local concentration of the factor slowly increasing, so that after some time the cell would sense a high concentration of the factor and 'think' that it was in the presence of a high density of cells secreting the same factor when it wasn't. From the above equations, as $t \rightarrow \infty$, the series terms vanish. For the cells on a thick permeable material, at large t, [x] approaches a constant value, and we calculated that this was far below the threshold concentration of a signal we were studying [26]. However, for cells in a thin layer of liquid, at large t, the $-\ln(r^2/4DT)$ continues to increase, and we used computation to show that for the factor we were studying, during physiological time scales, the [x] in the vicinity of an isolated cell secreting x would be well below the threshold for activity [26].

2.4. Computational Approaches to Understand how Cells Sense the Location of Other Cells

As described above, the simplest way to allow cells to sense where the bulk of the cells in a microbial community are, and thus how to move away from the community, is to have all the cells in the community secrete a chemorepellent (Fig. 1 and Fig. 2A). However, there are other possible ways to let cells sense how to move away from the community. Using Monte Carlo methods to model the diffusion of a small (and thus easily diffusible) chemoattractant (in this case, cAMP) and a relatively slowly diffusing 60 kDa protein that inactivates the chemoattractant (Fig. 2B), we found that a protein could act as a chemorepellent in a wide variety of geometries by inactivating a chemoattractant, generating a chemoattractant gradient that is low near the source of the inactivating protein, and high elsewhere. The work in Fig. 2B used arbitrarily chosen secretion rates and enzyme kinetics, and diffusion coefficients for cAMP and a 60 kDa hydrolase in a thin layer of water on a flat 2-dimensional impermeable surface. The model started with a point source (origin) of the attractant and hydrolase on a 2-dimensional grid. At each time step of the model, the point source generated some number of attractant molecules and some number of hydrolase molecules. At each grid square, including the origin, each molecule of the low molecular mass attractant had a high probability of moving to an adjacent grid square in a random direction; each molecule of the hydrolase had a lower probability of moving to an adjacent grid square. Then

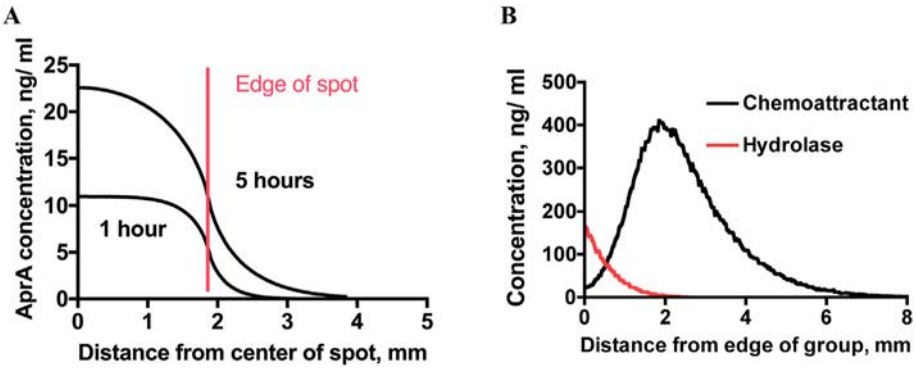

Fig. 2. Examples of diffusion calculations. A) Concentrations of a factor such as AprA secreted by all cells in a community as shown in Fig. 1, for the indicated times after the cells have started secreting the factor. B) More complex concentration profiles cannot be easily solved in closed form, but can easily be done with Monte Carlo approaches. In this example, all the cells in a community secrete a fast-diffusing chemoattractant, and a slowly-diffusing hydrolase that breaks down the chemoattractant. At each location of the hydrolase, at each moment, a molecule of the hydrolase has some probability of moving is a random direction, as well as a probability of breaking down a molecule of the attractant that depends on the local concentration of the attractant. Because of the stochastic nature of these probabilities, the graph shows some noise.

at each grid square, for each molecule of the attractant, there was a probability proportional to the number of hydrolase molecules at that grid square that the attractant molecule would be destroyed and removed from the count of attractant molecules at that grid square. Using actual diffusion coefficients, enzyme kinetics, and secretion rates, one could then play with the parameters and determine if such a mechanism could be feasible. As with the diffusing chalones, adding variable secretion rates, variable breakdown rates, and boundary conditions could be used to make such models more accurate.

2.5. Monte Carlo Simulations Predicted how a Stream of Cells Can Break into Groups

A basic question in developmental biology is how cells can sense the size of a group or tissue. When *Dictyostelium* cells starve, they aggregate into groups of roughly 2×10^4 cells. However, antisense repression or homologous recombination disruption of the gene *smlA* causes cells to form large numbers of small aggregates [7,31–37]. We found that the *smlA* phenotype is due to these cells oversecreting a factor that reduces group size. We purified the factor and named it Counting Factor (CF). CF is a complex of proteins that is secreted by developing wild-type cells. Deletion of any of the components of CF causes aggregation streams to not break up, resulting in the formation of huge fruiting bodies. I was baffled by how a stream of cells could break into groups, and I wrote computer simulations of streams and played with various parameters [38,39]. The simulations predicted that if a secreted factor such as CF increases random cell motility and decreases cell-cell adhesion, the streams break apart (Fig. 3). Experiments then showed that the predictions were correct. In mutants with no CF activity, random motility is low and cell-cell adhesion is high, and streams stay intact even if there are too many cells in the stream [38,39]. With high CF (*smlA*$^-$ cells), high random motility and low cell-cell adhesion cause streams to break. Decreasing cell-cell adhesion with antibodies against adhesion proteins caused streams to break excessively, while decreasing motility caused streams to stay intact [38,39]. This was an example where computer simulations led experiments, rather than vice versa. Adding variable secretion rates, variable breakdown rates, boundary conditions, and different initial distributions of cells could be used to make such models more accurate.

Fig. 3. Example of a Monte Carlo simulation used to understand how a stream of aggregating *Dictyostelium* cells can break up into groups. Dots represent cells. A) shows an initial stream, B) the stream after cells have dispersed due to random motility being, on average, stronger than cell-cell adhesion, and C) cells in the dispersed stream, after decreasing their random motility strength relative to the strength of cell-cell contacts, tend to aggregate back into groups.

2.6. Computational Work on Microbial Communities Led to Potential Therapeutics

As described above, computational approaches were an integral part of work to understand how cell-density sensing signals and chemorepulsion signals help cells in microbial communities exchange information. An attempt to determine if human white blood cells use a chalone mechanism similar to the *Dictyostelium* cell-density sensing mechanisms led to the identification of a potential therapeutic for fibrosing diseases that recently showed better efficacy in pulmonary fibrosis patients than current standard of care [40–44]. Computation of a predicted structure of the *Dictyostelium* chemorepellent AprA using I-TASSER [45] identified a human protein called DPPIV as a potential orthologue [46,47]. DPPIV was found to be a chemorepellent for human and mouse neutrophils, and in mouse models of the neutrophil-exacerbated diseases acute respiratory distress syndrome and rheumatoid arthritis, local application of DPPIV was able to ameliorate inflammation by moving neutrophils out of the area of inflammatory damage [46,48]. Computational work on signaling in microbial communities thus helped to transition this work to potential therapeutics.

3. Conclusion

As illustrated by the above examples, computational work has played a key role in our understanding of cell-cell communication in microbial communities, and this has, in turn, led to potential therapeutics for several different diseases.

Declaration of Conflict of Interest

None.

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