Noise-driven Cell Differentiation and the Emergence of Organization

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One of the major transitions in evolution is the monumental step from mere unicellularity into the brave new world of multicellularity. To understand this feat, one has to fathom two main characteristics of multicellular organisms that appeared during this transition: namely, differentiation and self-organization. Any explanation concerning this major transition should involve mechanisms that can simultaneously explain the marvelous intricacies manifest in the aforementioned characteristics, and an account of the evolution of such traits. Here we propose a noise-driven cell differentiation (NDD) model. Stochasticity is the raw material that fuels this model and this reliance on noise, in place of a more mechanical approach, that makes it a suitable model, which explains differentiation and self-organization and shed some light on the possible evolutionary origins of these biological innovations. To test our model, we utilize a model of biofilm formation. We show that the behavior of this simple model of cell differentiation and organization is in concert with the NDD model.

cell fate | stochasticity | self-organization | bi-stable switch

The traditional idea of a living cell where every organelle, every reaction, and every interaction is part of a clock-like order has long been shattered by the understanding that biological systems usually struggle to function in noisy environments. One might consider life to be an uphill battle against the forces of chaos, where disarray is the norm and spheres of order – i.e., biological systems – are rarities that are astronomically unlikely to appear in the first place. In this view, the existence of noise is the failure of natural selection to fully suppress this capricious force. It is for the same reason that selection cannot reduce the fidelity of replication beyond a certain threshold; the biological cost of increasing fidelity simply becomes too high at that point (1).

A different view has recently gained some grounds (2–5). In this view, biological systems that regulate and utilise the noise can have higher fitness under certain circumstances. Had biological systems been utterly deterministic, adaptation – i.e., the emergence of a new phenotype or a change in the gene expression pattern to utilise a new food source – in an isogenic population, without new mutations, would have been impossible. In reality, noise in the cell can result in beneficial non-genetic diversity in otherwise genetically homogenous populations – e.g., cyanobacteria (6) and yeast (7). But what mechanism can account for the presence of phenotypic diversity amongst daughter cells that are genetic clones of each other? Is it possible for a stochastic mechanism to explain the non-genetic diversity? Even if such stochastic explanation were offered, how could this explanation possibly account for the proportion, the arrangement, and the organization of non-genetic phenotypes in the population?

We propose that a stochastic model can provide us with a satisfactory explanation of how organization can emerge from chaos. Our approach is in keeping with the recent emphasis on the importance and the prevalence of noise in biological functions, specifically cell fate(2, 4, 8).

Our model of cell differentiation, henceforth referred to as the noise-driven differentiation (NDD) model, accounts for the peculiarities of this biological phenomenon by weaving noise into an explanation of cellular behaviours at the time of differentiation. While on the surface, our approach might seem lofty and even radical, the model discussed in this paper is patently parsimonious when it comes to the mechanisms requisite for its operation. The NDD model rests upon certain assumptions about biological systems:

1 **Noise, resulting from a plentitude of sources, is an inseparable part of a living cell:** There is a plethora of phenomena within a cell that can contribute to its intrinsic noise – e.g., transcription regulation, transcription factor binding to the DNA, RNA processing in eukaryotes, translation, post-translational modifications, protein complex formation, protein and RNA degradation, etc (9–11). For example, Mustonen et al. (12) looked at the expression levels of 12 genes expressed in response to the presence of copper in the environment in the earthworm *Dendrobaena octaedra*. The 5 earthworms used by Mustonen et al. (12) were clones so the observation that there was variation in the levels of expression amongst the 12 genes of interest between the clones can only be

Significance Statement

The ability of cells to beget different types of cells (differentiation), and the capacity of the cells to organize in an orderly fashion are innovations that led to the emergence of multicellular life forms. The molecular mechanisms used to explain the complexity of these phenomena makes it hard to understand them in an evolutionary context. Here the authors present a parsimonious model which attempts to explain differentiation and self-organization by considering the noisy environment that exists inside and outside a living cell.

MS designed research; RT contributed to the initial idea; HS and CP performed research; AK wrote the manuscript; HS and CP contributed to the methods section; MS and AK contributed to the introduction and the discussion; HS analyzed the data; HS and AK visualized the results. All authors read and approved the final manuscript.

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interpreted as the non-genetic noise inherent in gene expression. Single-cell level measurements of gene expression (13) further cements the notion that cells are intrinsically noisy when it comes translating its genotype into phenotype. Maamar et al. (14) studied the relationship between the noisy expression of a protein (ComK) and the ability of *Bacillus subtilis* to uptake DNA form the outside. They showed that the variation in ComK expression in genetically identical cells results in the emergence of a subpopulation of competent –i.e., able to uptake external DNA– cells. They further illustrated the causal relationship between the intrinsic noise related the gene expression and the observed phenotypic diversity by manipulating the efficacy of transcription and translation of ComK. They also showed that lower intrinsic noise results in fewer competent cells in the population. This phenomenon is not limited to "simple" prokaryotes; Chang et al. (15) showed that stochasticity in gene expression in a stem cell marker (Sca-1) in mouse progenitor cells determines the fate of these cells. In addition to the intrinsic noise, extrinsic noise, which stems from random fluctuations in the environment (e.g., temperature and pH), can also affect gene expression (16, 17). (component #1)

2 **A cell does not differentiate, but daughter cells are born differentiated:** Thus far, two categories of solutions to the problem of cell differentiation have been proposed: the first category consists of models that rely on cell-cell communication (reviewed in 18) and the second category relies on asymmetric cell division (reviewed in 19). The research project within the confines of the former category is mainly a quest to find the building blocks of the apparatus that makes the specific kind of cell-cell communication, purportedly needed for cell differentiation, possible. The latter category, on the other hand, presumes the asymmetric cell division to result in differentiation. Hitherto unknown and often complicated mechanisms have been proposed to explain the asymmetric distribution of fate-determining factors during cell division (20–22). Both categories are quintessentially Hobbesian in nature (23), since they rely on mechanical interactions at the cellular level. While we agree with the importance of the asymmetric cell division, it seems to us that a stochastic model of differentiation, like the NDD model, negates the need for proposing new mechanisms yet be discovered. In this model, we adopt the view that stochastic processes result in differentiated cells due to the distribution of key proteins, instead of cells differentiating by receiving signals after they are born. (component #2)

3 **Stochastic partitioning of cytoplasm during cell division and the random distribution of molecules in the cytoplasm determine the cytoplasmic contents of the daughter cells:** Although, for the sake of simplicity, cell division plane is usually depicted as being situated in the middle of the cell, we know this depiction to be a pedagogical simplification. One can point out to the formation of polar bodies, a rather extreme example of an unequal cell division. While we do not necessarily expect cell divisions to be generally as asymmetrical as in the example of polar bodies, one would expect a range probabilities governing the position of the plane along which cells divide (reviewed in 24–28). The displacement of the division plane relative to the middle of the cell can result in an unequal distribution of cell content between the daughter cells. The stochasticity provided by an unequal cell division is independent of the distribution of the molecules in the mother cell, i.e., the resulting stochasticity holds even if molecules are homogeneously distributed within the cell. In addition to the noise generated by the stochastic positioning of the division plane, the random distribution of molecules, including the proteins, within the cell contribute to the the unequal distribution of cell content between daughter cells. The stochastic effects are more pronounced for molecules that exist in smaller numbers in the cell(29**?** –31). Such effects can result in the unequal distribution of key factors that determine cell fate. (component #3)

4 **Cell fate is determined by a switch.** The notion that cell fate is determined by a switch is best illustrated in the now famous case of the λ phage. As first shown by Shea and Ackers (32), the process by which the phage decides to integrate into the host's genome – i.e., lytic – or to replicate copies of itself in the cell until it bursts open – i.e., lysogenic – can be explained by a stochastic switch which makes that portentous decision in a probabilistic fashion, while taking into account the presence of certain key factors, e.g., *c*I and cro. As shown by Perez-Carrasco et al. (33) using a mathematical model, the intrinsic noise in the cell (component #1) can influence the decision made by the toggle switch. Similar switches are not limited to simpler organisms, but can be find in a variety of taxa (reviewed in 2). (component #4)

5 **The interaction between the building blocks of the switch determine its bias.** Using a bistable switch in the λ phage would not have persisted as a winning evolutionary strategy if its behaviour was indistinguishable from that of a homunculus throwing a fair coin to decide if she wants the virus to follow through a lytic life cycle or a lysogenic one. Quite the contrary, as the phenomenological model by Shea and Ackers (32) vividly illustrates, the strength of interactions within the switch make one outcome more likely than the other, e.g., as the concentration of *c*II protein increases, so does the likelihood of the reactions suppressing the activation of promoters relevant to the onset of the lytic trajectory, which in turn, tilts the scale away from lysis towards lysogeny. Thus, a bistable switch is akin to a coin, whose fairness is determined by the interaction between its building blocks. (component #5)

6 **The robustness of the switch is the result of a complex network of interactions.** Although the switch used to make decisions at the cellular level is stochastic, it has been shown that a toggle switch is immune to environmental fluctuations, until there is a significant increase in those fluctuations (34). Sharifi-Zarchi et al. (35) took advantage of the gene expression profiles of 442 mouse embryonic cells to construct a network of key transcription factors (TFs). While a regulatory circuit with two TFs could explain differentiation, Sharifi-Zarchi et al. (35) reasoned that such a simple switch is susceptible to

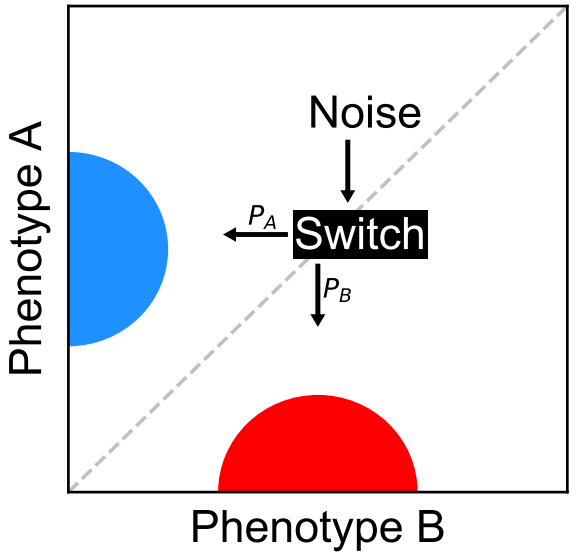

Fig. 1. A schematic representation of the NDD model. In a bistable switch, two states are available: A and B. The switch choose each of these states with probability, P_A and P_B, accordingly. The likelihood of a switch choosing one state over the other depends on the interaction between the switch and the noise. In the NDD model, illustrates the nature of the switch and it is interaction with the varieties of noise, resulting in a noise-driven explanation of phenotypic differentiation (See the components of the NDD model).

mutations. To construct a robust switch, they built a circuit with two clusters of TFs with correlated expressions. Expectedly, the alternative switch, which involved more interactions, was much more robust, i.e., the phenotype of the switch was more impervious to mutations. We would expect different levels of robustness for a switch, given its biological importance in evolution. (component #6)

7 **All the information needed to construct the switch is genetic.** The genetic information is all a living cell needs to construct the switch. The reason for this assumption is simple: if we were to imagine the emergence of an array of proto-switches: some of these switches had all their biological competence hardwired in their genome, including their sensitivity to noise, while others relied on non-genetic factors ("environment" in its broadest sense). Even though some members of the latter category are more competent than the average member of the former, their evolution would be problematic. Since their phenotype is mostly explained by the environmental variance, directional selection cannot improve them, and even if they somehow made the leap form proto-switch to switch, natural selection simply would not be able to maintain their competence by stabilizing selection because their genetic variance is already low and their variation is mainly due to environmental variance (our reasoning here is based on Fisher's fundamental theorem of natural selection (36, p.34)). (component #7)

The components #1-7 are sufficient to generate a population of cells with different proportions of two phenotypes, figure 1. While this kind of fate determination is adequate vis-à-vis primitive cells with no organization, it does not allow the emergence of multicellularity. An additional component is necessary to explain this major transition from mere phenotypic differentiation to self-organization in a body of a multicellular organism.

8 **Cell fate is determined by its location and its environment.** What we mean by self-organization is the phenomenon in which a cell differentiates in a way that results in a non-random distribution of cell types in the population. Even in a rudimentary multicellular organism like *Volvox*, cells cannot simply aggregate willy-nilly in order to make up the body of the organism. Instead, cells have to organize in a way that would result in an empty ball of somatic cells, with germline cells floating within this cellular sphere (37). For this level of self-organization to occur, the toggle switch determining cell fate should, in addition to being swayed by the intrinsic factors, be influenced by its neighbors. (component #8)

The desiderata listed above are backed by theoretical and experimental studies. We propose that these components, when taken together, provide us with a framework which includes the necessary conditions required for a cell to differentiate. What makes the NDD model attractive is its stalwart adherence to William of Occam's philosophy: the NDD model does not require any "wonder tissue" – i.e., a new molecular mechanism – to explain the process of differentiation.

In this study, to test the veracity of the NDD model, a computational model of biofilm formation has been used, Kreft et al. (38, 39). The biofilms are aggregations of bacterial cells attached to a surface (reviewed in 40). The phenotypic differentiation and self-organization in biofilms have been widely studied (41, 42). Modelling the differentiation in biofilms allows us to thoroughly test if the components of our NDD model are necessary and sufficient to replicate the collective behavior seen in biofilms in nature.

Materials and Methods

The NDD model was tested using a computational model of biofilm growth. An individual-based model (IBM) was implemented to simulate biofilm growth according to Kreft et al. (38, 39). A complete description of this model can be found in (43–45).

The individual-based model simulation. In the IBM simulation, the biofilm is made up of cells, where each cell is a circular particle defined by its state variables – e.g., spatial position, size, and phenotype. The simulation geometry is a circle with the radius of R and no flux boundaries.

Phenotype. Depending on the amount of its transcription factors, a cell can have two phenotypes, A and B; each representing the two possible cell types, as shown in figure 1 –i.e., stationary and motile– in the biofilm. In these simulations, the abundance of protein X leads to phenotype A, with which we start the simulation, and the dominance of protein Y results in the cell to have phenotype B. In fact a negative feedback loop influences the cell decision making process. Two negatively coupled repressors mutually inhibit the expression of the gene that encodes the other repressor. The rate of this mutual repression is given with a toggle switch (component #4) in the form of Hill function. This negative feedback loop

results in two stable steady states, hence implies non-linear approaches. Nonlinear differential equations govern the changes in the number of the repressor proteins, X and Y;

$$\frac{dX}{dt} \sim \beta \frac{S^n}{S^n + Y^n} - \gamma X \quad ,$$
$$\frac{dY}{dt} \sim \beta \frac{S^n}{S^n + X^n} - \gamma Y \quad . \qquad [1]$$

Here, β is the maximum production rate and n is the Hill coefficient, which represents the degree of competence and S stands as the dissociation constant. γ is the degradation rate of TFs. This bistable regulatory network has two attractors corresponding to its stable steady states. Based on the amount of proteins at the cell division time, the cell can be in the domain of each attractors, hence its fate is determined. Depending on the intensity of inhibitory effects of TFs (through the values of constants in the Hill function), the two domains of attractors could be equal or not (component #5).

Cell growth. Simulations were started with a single bacterium with phenotype A and followed the biofilm growth for some generations. Bacteria use nutrients transported only by diffusion and grow in size. We assume here that there is only one nutrient that limits the cell growth rate. The cell growth (R_H) starts from the center of the circular area and it follows a Monod type kinetics as a function of the concentration of substrate, C_s, and the concentration of heterotrophic biomass, C_H:

$$R_H = \mu^{max} \frac{C_s}{K_s + C_s} C_H, \quad [M_H L^{-3} T^{-1}] \quad . \qquad [2]$$

μ^{max} is the maximum specific growth rate of biomass, K_s is the half-saturation constant for the substrate. Accordingly, the consumption rate of substrate (R_s) is proportional to the biomass production rate:

$$R_s = -Y_{sH} R_H, \quad [M_s L^{-3} T^{-1}] \quad , \qquad [3]$$

where Y_{sH} is the yield coefficient. We numerically solve a diffusion-reaction equation, according to the mentioned reaction rates, to find the concentration of the solute species at the position of bacteria in the biofilm colony;

$$\frac{\partial C_s}{\partial t} = D \nabla^2 C_s + R_s \quad , \qquad [4]$$

where D is the diffusion coefficient of substrate in the biofilm colony.

Biofilm growth algorithm. Each iteration in the simulation can be divided into four steps:

1. *Cell growth*: In this step, bacterial cells consume nutrients from the environment, according to equation 2, and grow in size. Simultaneously, the cytoplasmic content of each cell fluctuates in a stochastic fashion. This is the fact implied by component #1. The repressor proteins inside the cytoplasm interact with each other and their numbers, X and Y are updated; however, because of their low copy number, their fluctuations are treated by stochastic dynamics for discrete values. Compared to the cell growth rate, it was assumed that the cytoplasmic content reaches the steady state in a very short period of time. Hence, in each simulation step, using the Gillespie algorithm (46) which is a stochastic simulation algorithm for systems with low copy numbers, the numbers of proteins X and Y will be updated. According to this algorithm, a probability will be assigned to all the possible biochemical reactions in the system; based on these probabilities, the reactions have the chance of occurrence. In the process of updating the cytoplasmic content, for every protein (X or Y), there exists a reaction that produces it and another reaction that destroys it. The probability for the production of proteins follows the first term on the right hand sides of the Eq. (1), as a Hill function. As the number of protein X increases, it further represses the production of protein Y and vice versa. To reduce the number of protein X, the destruction reaction for this protein happens according to $-\gamma X$. In every step of the Gillespie algorithm, one of the above reactions occurs and the time will be updated. The process continues until the number of proteins reach a steady state.

2. *Cell division*: Even after the number of proteins in a cell reaches the steady state, the cell continues to grow. The growth stops only after the cell reaches a critical size. At this point the cell divides into two daughter cells. The content of the mother cell is distributed among her daughters according to a uniform distribution. The position at which cell division occurs is randomly chosen based on a beta distribution (component #3). At the time of birth, the phenotype of each newborn cell is determined (component #2), based on the cytoplasmic contents (genetic information) inherited from the mother cell (component #7) and it will not change during cell's lifespan. The fate of each daughter cells is determined by the number of key proteins, X and Y, at the time of birth. During the cell growth, the number of each protein has a stochastic trajectory in the domain of its attractor and finally it will reach its steady state. There is no transition to the domain of the other attractor, hence, no change of phenotype during the cell growth (component #2). The only transition occurs on the division time. In this model, phenotypic change is reversible, meaning that the phenotype can change between the two possible states over generations.

3. *Relaxation*: After a cell divides, the cells comprising the biofilm push each other outwards to make room for the new daughter cells, Kreft et al. (39).

   Simulation proceeds by repeating the steps #1-3. It is worth noting that, without considering self-organization, the process described above would result in a disordered blob of cells.

4. *Self-organization*: To involve the self-organization phenomena in the process of cell maturation based on component #8, cells secrete some signaling molecules, with concentration C_c , which influence the production rate of proteins through propensity functions as well as the cell fate. The signaling molecules, which have a lifespan, diffuse in the medium according to the following reaction-diffusion equation,

$$\frac{\partial C_c}{\partial t} = D_c \nabla^2 C_c + k_{cp} Y - k_{cc}(X + Y) - k_{cd} C_c \quad . \qquad [5]$$

Here, k_{cp}, k_{cc} and k_{cd} represent, respectively, the rate of production, consumption and decay of the signaling molecules and D_c is their diffusion coefficient of the signals. In these simulations, the secreting cells are those with phenotype B (black color); hence, the production of signaling molecules is proportional to the amount of Y proteins within B cells. Since both phenotype consume these molecules, the consumption depends on the number of both X and Y TFs. Simulation starts with a cell of phenotype A and continues for some generations of bacteria. The cell's cytoplasm is updated stochastically according to Gillespie algorithm, based on the reactions rate in equation (1). The stochastic nature of the cell growth process results in the emergence of cells with phenotype B. Then, the secretion of signaling molecules by B cells, which diffuse in their environment, attenuates the production of protein X, responsible for phenotype A and augments the production of protein Y; as a consequence, their surrounding cells would have less chance of producing protein X and their offsprings have less chance of being in the domain of attraction of protein X, hence becoming a B type, figure 3.

Results

The overall behavior of the biofilm growth simulation, as depicted in [movie SI1 (a 3D simulation)] demonstrates the principles of our framework – that is, the stochasticity results in phenotypic heterogeneity as the biofilm grows in size. To further illustrate how each source of noise affects the cell differentiation, we focused on each source separately in our simulations (discussed below).

The stochastic positioning of division plane and the stochastic distribution of key proteins affect differentiation. One source of intrinsic stochasticity stems from the random positioning of the division plane. This factor would disproportionately influence the number of molecules that exist in low numbers within cytoplasm. We postulate that the determinants of cell fate are low in numbers and thus greatly affected by the stochasticity. To demonstrate this phenomenon, we allowed for different levels of variance in the position of the division plane respect to the mid plane of cells, while fixing the distribution of proteins that determine the cell fate – i.e., the proteins are distributed in the cytoplasm such that the exact number of proteins found at any distance from the division plane is a fixed number.

Starting from a cell with phenotype A in which the concentration of protein X dominates over protein Y and considering the mentioned sources of noise, the population heterogeneity, emergence of phenotype B, was traced over more than 15 generations. The results are shown in figure 2, as the phenotypic diversity in the population. The curves have different number of protein X in the steady state, in increasing order from top to the bottom.

When the division plane is situated in the middle of the cell, we observe no differentiation (A cells persist and no B cell is generated). As the bias increases, so does the proportion of B cells. This phenomenon is also dependent on the number of proteins since such bias is only relevant when the number of proteins is relatively low. In fact, excluding every source of noise and having large copy numbers of TFs in the mature cell, the new born cells, after the division, have almost the same density of genes as their mother (deviations in their population has minor effects); hence, in the phase space, they will be in the same domain as the mother and their fates will be identical to her. This can be seen clearly in the lower curves in the figure 2, in which phenotype is in most cases unchanged. However, for low copy number of TFs, the difference between TF numbers in two daughter cells becomes more prominent and can even lead to different cell fates (top curves in figure 2). Therefore, in this case, it is possible to have heterogeneity in the population in the absence of any other noise, i.e., cells with low TF numbers are heterogeneous even with no variance in division-plane displacement (figure 2A).

Having the TFs uniformly distributed within a cell only increases the chance of differentiation, since now in addition to the noise from the positioning of division plane, the key proteins are now stochastically distributed as well (figure 2B).

Signal from other cells can created a biofilm with order. In our biofilm model, B cell can release signals in the environment. These signals diffuse at an slower rate than the nutrients in our biofilm model, and consequently have a very short radius of influence. The absorption of these signals by other cells in the biofilm affects the concentrations of proteins involved in the switch – that is, switching to the phenotype B during cell division becomes more likely. As shown in [movie SI2], when we add this environmental signaling to the biofilm, the cells organize in a non-random fashion, a stark contrast to the random heterogeneity observed before [movie SI1].

Figure 3 qualitatively represents a visual understanding of NDD results. It shows the bacterial community in a 2 dimensional simulation area after more than 15 generations. In panel A, the variance in the stochastic positioning of the division plane as the only source of noise increases from left to right. It can be seen that the heterogeneity in the population increases as well by the presence of new phenotypes (cells in black). Addition of another noisy phenomenon, uniform distribution of TFs in the cytoplasm also amplifies the heterogeneity, as in the figure 3B. In panel C, development of an organized community as a result of signaling molecules is apparent (group of black cells); from left to right, by increasing the effect of these molecules, a greater community takes shape. The organization observed in panel C will increase over time and the community of black cells will develop; however, some small populations of these cells in panel A or B will probably vanish as they are not stable.

Discussion

Molecular processes in the cell are noisy events that result in varying degrees of heterogeneity. Taming this inherent noise is vital for the emergence and the continuation of life. In fact, life can be defined as a system with the capacity to control noise.

The phenotype of a cell is generally stable, but during cell division, this cell can produce daughter cells with different phenotypes via symmetric or asymmetric cell division. The resulting non-genetic phenotypic diversity is a way to achieve adaptation in a fluctuating environment by producing phenotypically divers offspring without any need for genetic change.

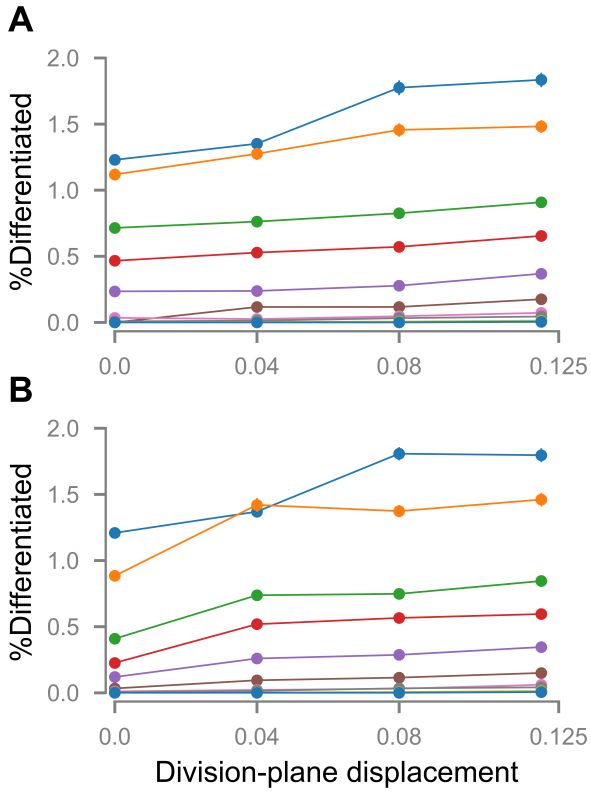

Fig. 2. The stochastic positioning of the division plane and random distribution of TFs in the cytoplasm, as intrinsic noise, affects the none-genetic phenotypic diversity (component #3). The phenotypic diversity is represented by the proportion of cells with the phenotype B related to the total number of cells in the population. In panel A, the only source of noise is the stochastic positioning of the division plane, while panel B shows the phenotypic diversity as a result of both sources of noise. In each panel, the curves indicate different amounts of protein X in the mother cell; from top to bottom, respectively, $X = 10, 15, 20, 25, 30, 35, 40, 45, 55, 65, 100$. The results are average over 1000 samples.

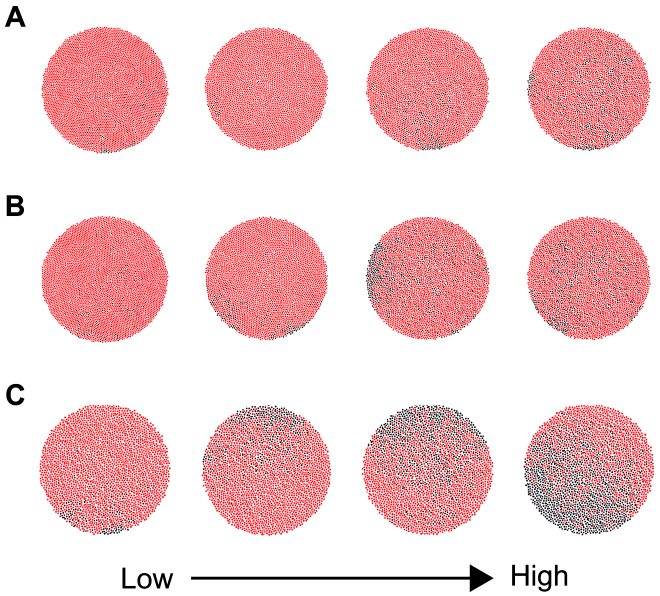

Fig. 3. Emergence of a community of cells with new phenotype as a result of the noise in the distribution of TFs (A), the variance in the position of the division plane (B), or the secretion of signals from B-type cells (C). The red dots represent A-types cells and the black ones represent the B-type cells. Each circle is the final state of a single run of our stochastic model with the given parameters. For each row, the parameter in question –e.g., noise in the distribution of TFs in row A– increases from left to right, as indicated by the arrow. The average amount of protein X in the mother cell is 25 and the radius of the area $\approx 50\mu$.

Given the variety of sources of noise, the cell fate determination can be a stochastic process. One can imagine a few genes involved in cell fate determination, where the noise in the cell affects the proportion of daughter cells born with a certain phenotype. The ability to change the phenotypic proportion of daughter cells via a stochastic mechanism, which is also tunable, is a superb strategy to outcompete rivals bereft of such gifts.

The ability of the cells to differentiate into different types was the crucial step that enabled the ancient solitary cells to leave the primordial soup behind and evolve into the vast array of specialized cells we see today. As Queller and Strassmann (47) point out, there are different shades of organismality –i.e., the ability for components to work together with little conflict among them–, each shade resulting from the affinity of the members of the system to cooperate versus the temptation to cheat. We can sidestep the problem of conflict since in prokaryotic multicellularity, e.g., biofilm, and in most truly multicellular eukaryotes, the cells are highly related, thus lowering the probability of cheating (48). Without tangible levels of conflict, multicellularity as a trait becomes patently advantageous. In their seminal work, Maynard Smith and Szathmáry (49) considered two possible mechanisms to account for the emergence of cell differentiation: one relies on the presence of determinants that prohibit the stem cell to differentiate, and the other postulates the cell-cell contact as a mechanism that determines cell fate. While these suggestions account for how the multicellularity might be sustained, they do not explain how this major evolutionary transition could have occurred in the first place.

It is easier for cell differentiation to evolve via the emergence of a switch, rather than the lass plausible path that involves the evolution of a clockwork mechanism.

According to the NDD model, the emergence of early stages of multicellularity only requires the evolution of a suitable switch – the rest of the necessary ingredients needed for the transition into self-organization is provided by the stochastic elements affecting the switch. The major transition from unicellularity to multicellularity –i.e., from phenotypic diversity in a population to ordered and stable spatial heterogeneity– only requires one more step: the evolved switch should be simply affected by the signal(s) released by its neighbours (components #8). The spatial information received in this way would bias the switch such that the population-level organization is retained.

One of the quintessential aspects of our model is its population-level perspective. Population-level thinking is one of the main points of the evolutionary theory, and bringing it to explain a cellular phenomenon can lead us to reap valuable insights. While a population of cells has, on average, certain

properties relevant to differentiation, e.g., the mean number of key proteins, the average position of cell division plane, and etc. these average values do not tell the whole story. Instead, the variance in these values, i.e., the non-genetic variation present amongst individuals, is the key to the differentiation (as observed in studies such as in (15)). This "noise" in the population is essentially the fuel that propels cellular differentiation, be it in the reversible differentiation in prokaryotes or the more complicated irreversible ones in higher organisms. We believe that this population-level vintage point is the necessary tool to understand this otherwise mind-boggling biological process. Without this perspective, the task of explaining such a seemingly fine-tuned process devolves into an attempt to come up with complex cellular interactions that would make climbing this improbable biological mountain feasible.

Suzuki et al. (50) utilized a model of differentiation where oscillatory protein expression gives a stem cell its quintessential property. According to their model, the loss of synchronous oscillation after cell division results in irreversible cell differentiation. In their criticism of a noise-driven alternative to their model, Suzuki et al. (50) proposed that it would be unlikely for a noise-driven model to maintain the exact levels of stochasticity needed for the model to result in the desired proportion of differentiated cells versus stem cells. It seems that conclusion follows from a non-evolutionary perspective, since it is easy to imagine negative selection keeping a genetic switch just sensitive enough to result in a correct differentiation pattern vis-à-vis the biological fitness. Furthermore, this evolutionary explanation can be easily tested: if we were to measure the effect of selection by measuring the proportion of non-synonymous substitutions to synonymous ones ($\frac{dN}{dS}$, following McDonald and Kreitman (51)), we would expect $\frac{dN}{dS} < 1$, which signifies the effect of negative selection on the genes involved with the functionality of the switch.

Suzuki et al. (50) point out that a noise-driven model can only produce reversible differentiation. While the NDD model as described here only explains the phenotypic differentiation in prokaryotes, which is a reversible phenomenon, it seems that changing the bi-stable switch to a tri-stable one could remedy this issue and explain the irreversibility of differentiation observed in eukaryotes, since it has been shown to increase the strength of attractors (52). In the NDD model, organization is created and maintained by the signals released by the cells. Upon receiving the signal, the switch will be more likely to produce a certain type of cells over the other type(s) during division, since the switch now "knows" where the cell carrying it is located relative to the neighboring cells. This model of organization dispenses with any complicated mechanism needed for cell migration that would otherwise be required for the phenotypic assortment of cells. The eukaryotic flavor of phenotypic differentiation with its irreversibility became possible by the emergence of a tri-stable switch.

The NDD model can be used wherever there is cell division and differentiation. The differentiating cell can be a prokaryotic cell, able to divide into daughter cells with dissimilar, and reversible, phenotypes or a eukaryotic cell undergoing irreversible differentiation, without the need for one or a few complicated mechanisms. The transition from single cells into the brave new world of multicellular entities could have been the result of a mechanism very much akin to the NDD model. This transition was possible because the bias of the switch can be affected by the neighboring cells. The NDD model paints a simple and elegant picture of differentiation and organization, from prokaryotes to eukaryotes. The biofilm was chosen as model of phenotypic heterogeneity where the components of our model could have been tested. Our model biofilm growths allows for a relatively realistic depiction of a process that results in phenotypic differentiation in a population. However, we believe that, with few changes, the NDD model can be applied to other biological systems as well.

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