Noise-driven Cell Differentiation and the Emergence of Organization

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Abstract One of the major transitions in evolution is the step from unicellularity into the brave new world of multicellularity. To understand this feat, one has to fathom two main characteristics of multicellular organisms: 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. The reliance on noise, in place of a more mechanistic approach, makes the NDD model a more suitable approach to explain differentiation and self-organization. Furthermore, our model sheds some light on the possible evolutionary origins of these biological innovations. To test the NDD model, we utilize a model of biofilm formation. The behavior of this model of biofilm formation is in concert with the NDD model.

Introduction

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 pandemonium, where disarray is the norm and spheres of order – i.e., biological systems – are rarities that are unlikely to appear in the first place. In this view, noise is a nuisance that natural selection always attempts to eliminate. It is for the same reason that selection cannot increase the fidelity of replication beyond a certain threshold; the biological cost of increasing fidelity simply becomes too high at that point (***Kimura, 1967***).

A different view has recently gained some grounds (***Balázsi et al., 2011***; ***Chalancon et al., 2012***; ***Huang, 2009***; ***Losick and Desplan, 2008***). In this view, biological systems that regulate and utilize 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 utilize a new food source – would have been impossible without the emergence of new mutations. In reality, noise in the cell can result in beneficial non-genetic diversity in otherwise genetically homogenous populations – e.g., cyanobacteria (***Wolk, 1996***) and yeast (***Paliwal et al., 2007***). 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?

Here, we propose that a stochastic model can provide a satisfactory explanation of how organization can emerge from noise. Proposing a stochastic model of cell differentiation is not an entirely novel concept; for instance, Kunihiko Kaneko and his colleagues have produced an impressive body of work on this very subject (reviewed in ***Suzuki et al., 2011***), but we argue that our approach differs from theirs and similar ideas in certain important aspects (discussed below). This approach is in keeping with the recent emphasis on the importance and the prevalence of noise in biological functions, specifically cell fate (***Balázsi et al., 2011***; ***Huang, 2009***; ***Kittisopikul and Süel, 2010***).

The model of cell differentiation proposed in this work, 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 behaviors at the time of differentiation. While on the surface, this approach might seem lofty and even radical, the model discussed in this paper is parsimonious when it comes to the mechanisms requisite for its operation. The NDD model rests on 8 components. Some can be regarded as facts, based on reliable empirical evidence from biological systems (components 1 and 3), while others are more accurately described as assumptions (components 2 and 4-8). The components of the NDD model are:

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 (***Kepler and Elston, 2001***; ***Ozbudak et al., 2002***; ***Elowitz et al., 2002***). Single-cell level measurements of gene expression further cements the notion that cells are intrinsically noisy when it comes translating its genotype into phenotype (***Sanchez and Golding, 2013***). ***Maamar et al.*** (***2007***) studied the relationship between the noisy expression of a protein (ComK) and the ability of *Bacillus subtilis* to uptake DNA form the environment. They showed that the variation in ComK expression in genetically identical cells results in the emergence of a subpopulation of competent cells, i.e., able to uptake external DNA.
   This phenomenon is not limited to ”simple” prokaryotes; ***Chang et al.*** (***2008***) 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 (***Lo et al., 2006***; ***Bumke et al., 2003***). (component #1)

2. **The fate of a cell is determined when it is born:** Thus far, two types 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 ***Wolpert, 2011***) and the second category relies on asymmetric cell division (reviewed in ***Rudel and Sommer, 2003***). 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 needed for cell differentiation. 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 (***Morrison and Kimble, 2006***; ***Betschinger and Knoblich, 2017***; ***Clevers, 2005***). Both categories are

quintessentially mechanistic in nature, 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 new mechanisms. 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, it is known 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 it is not necessarily expected the 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 a cell divides (reviewed in ***Margolin, 2000***; ***Monahan et al., 2014***; ***Pickett-Heaps et al., 1999***; ***Wu and Tzanakakis, 2012***; ***Bradshaw and Losick, 2015***). 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 the 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 unequal distribution of cell content between daughter cells. The stochastic effects are more pronounced for molecules that exist in smaller numbers in the cell(***Allen et al., 2013***; ***Huh and Paulsson, 2010***, ***2011***; ***Lee et al., 2013***). 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*** (***1985***), the process by which the phage decides to integrate into the host's genome – i.e., lysogenic – or to replicate copies of itself in the cell until it bursts open – i.e., lytic – 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.*** (***2016***) 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 found in a variety of taxa (reviewed in ***Balázsi et al., 2011***). (component #4)

5 **The interaction between the building blocks of the switch determines its bias.** Using a bistable switch in the λ phage would not have persisted as a winning evolutionary strategy if its behavior 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*** (***1985***) 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. (component #5)

6 **The robustness of the switch is the result of a complex network of interactions.** Mutational robustness can be defined as the ability of a phenotype to be viable in the face of mutations (***Gardner and Kalinka, 2006***). ***Sharifi-Zarchi et al.*** (***2015***) 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.*** (***2015***) reasoned that such a simple switch is susceptible to 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 from 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 (the reasoning here is based on Fisher's fundamental theorem of natural selection (***Fisher, 1930***, 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 the self-organization means 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 haphazardly 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 (***Kirk, 1998***). 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. It is proposed that these components, when taken together, provide us with a framework that 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 require no "wonder tissue"(***Dennett, 2014***) – i.e., a new molecular mechanism – to explain the process of differentiation.

To test the veracity of the NDD model, an individual-based approach has been used as a case study (***Kreft et al., 1998***, ***2001***). A complete description of this model can be found in (***Picioreanu et al., 2004***; ***Xavier et al., 2005a***,b). The biofilms are aggregations of bacterial cells attached to a surface (reviewed in ***Donlan, 2002***). The phenotypic differentiation and self-organization in biofilms have been widely studied (***Nadell et al., 2009***; ***van Gestel et al., 2015***). Modelling the differentiation in biofilms allows us to thoroughly test if the components of the NDD model are necessary and sufficient to replicate the collective behavior seen in biofilms in nature. Given our emphasis here on differentiation and self-organization, we did not test the evolution of robustness in the context of this model of biofilm growth (component #6).

Results

The overall behavior of the biofilm growth simulation, as depicted in movie S1 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 the simulations.

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. In this work, it has been postulated that the determinants of cell fate are low in numbers and thus, greatly affected by stochasticity.

To demonstrate this phenomenon, the position of the division plane was allowed to vary with respect to the mid plane of the cell. Starting from a cell with phenotype A, in which the protein X is dominant, the population heterogeneity –i.e.,emergence of phenotype B– was traced over 12 generations. The results are shown in figure 2. When the division plane is situated in the middle of the cell, there is no differentiation (A cells persist and no B cell is generated). As the variance in the cell-division plane increases, so does the proportion of B cells. This phenomenon is dependent on the number of proteins, since such bias is only relevant when the number of proteins is relatively low. In fact, with large copy numbers of TFs in a cell, its daughters will have almost the same density of TFs as their mother. Thus, they will be in the same domain as the mother in the phase space, and their fates will be identical to hers. This can be seen clearly in the lower curves in the figure 2, in which the phenotype is in most cases unchanged. However, for low copy numbers of TFs, the difference between TF numbers in two daughter cells becomes more prominent and can even lead to different cell fates. 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). Adding spatial fluctuation to the distribution of TFs within a cell increases the chance of differentiation, since in this case, in addition to the noise from the positioning of division plane, the key proteins are stochastically distributed as well (figure 2B).

Signal from other cells can created a biofilm with order

In this biofilm model, B cell can release signals in the environment. These signals diffuse at a slow rate and, consequently, have a very short radius of influence. The absorption of these signals by other cells in the biofilm affects the number of proteins involved in the switch – that is, switching to the phenotype B during cell division becomes more likely. As shown in movie S2, when this environmental signaling is added to the biofilm, the cells organize in a non-random fashion, a stark contrast to the random heterogeneity observed before [movie S1].

Figure 3 qualitatively represents a visual understanding of the results from the NDD model. It shows the bacterial community in a 2 dimensional simulation area after more than 12 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). Adding another source of noise, spatially-fluctuated distribution of TFs in the cytoplasm, amplifies the heterogeneity (figure 3B). In panel C, development of an organized community as a result of signaling molecules is apparent (group of black cells). 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 (movie S2).

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 characterized 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 diverse offspring without any need for genetic change. 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 gift.

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*** (***2009***) 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 (***Ostrowski and Shaulsky, 2009***). Without tangible levels of conflict, multicellularity as a trait becomes patently advantageous. In their seminal work, ***Maynard Smith and Szathmáry*** (***1995***) 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 less 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 from an ordered and stable spatial heterogeneity– only requires one more step: the evolved switch should be simply affected by the signal(s) released by its neighbors (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 the discussed 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 understand differentiation (as observed in studies such as in (***Chang et al., 2008***)). 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. (***2011***) 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.*** (***2011***) 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*** (***1991***)), 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.*** (***2011***) also 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 (***Ghaffarizadeh et al., 2014***).

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 one, 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. Such transition is 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 a model of phenotypic heterogeneity, where the components of our proposed model could have been tested. This model of 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.

Materials and methods

In the individual-based model, 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. It is assumed that the relative amount of two key transcription factors, X and Y, controls the cell types; hence, in this simulation, a cell can have two phenotypes, A and B, as shown in figure 1 –i.e., stationary and motile– in the biofilm. The dominance of protein X leads to phenotype A and the dominance of protein Y results in the phenotype B. In fact, a positive 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 represented in the form of a Hill function (***Gardner***

et al., 2000). This positive feedback loop results in two stable steady states, hence implies non-linear approaches- i.e., a toggle switch (component #4). Nonlinear differential equations govern the changes in the number of the repressor proteins, X and Y;

$$\frac{dX}{dt} = \frac{\beta}{1+Y^n} - X \quad ,$$
$$\frac{dY}{dt} = \frac{\beta}{1+X^n} - Y \quad . \tag{1}$$

Here, β is the effective rate of protein synthesis and n is the Hill coefficient, which represents the degree of competence. The number of repressors are represented in the unit of their dissociation constants and time is rescaled by degradation rate of proteins (***Gardner et al., 2000***; ***Carson and Cobelli, 2000***; ***Elowitz and Stanislas, 2000***). 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 (***Gardner et al., 2000***)), the two domains of attractors could be equal or not (component #5).

Biofilm growth algorithm

Simulations were started with a single bacterium with phenotype A and followed the biofilm growth for some generations. Each iteration in the simulation can be divided into four steps:

1. *Cell growth*: In this step, bacterial cells grow linearly in size. Simultaneously, the cytoplasmic content of each cell fluctuates in a stochastic fashion (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 numbers, instead of deterministic equations (Eq. 1), their fluctuations are captured by the Gillespie algorithm (***Gillespie, 1977***) as a stochastic dynamics for discrete values. According to this algorithm, a probability of occurrence will be assigned to every biochemical reaction in the system. Every protein (X or Y) is produced with a probability according to the first term on the right hand sides of the Eq. (1). As the number of protein X increases, it further represses the production of protein Y and vice versa. Every protein degrades according to its number. 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 reaches 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 normal distribution (component #3). At the time of birth, the phenotype of each newborn cell is determined based on the cytoplasmic contents (number of key proteins, X, and Y at the time of birth) inherited from the mother cell (components #2 and #7). 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. 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., 2001***).
   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 phenomenon in the process of cell maturation (component #8), cells secrete some signaling molecules, with concentration C_s, which affects the propensities in the Gillespie algorithm and, consequently, the production of proteins.

The signaling molecules diffuse in the medium according to the following reaction-diffusion equation:

$$\frac{\partial C_s}{\partial t} = D_s \nabla^2 C_s + k_{sp} C_B - k_{sc} \frac{C_s}{K_s + C_s}(C_A + C_B) - k_{sd} C_s \quad . \tag{2}$$

Here, k_{sp}, k_{sc} and k_{sd} represent, respectively, the rate of production, consumption and decay of the signaling molecules and D_s is the diffusion coefficient of the signaling molecules. C_A and C_B respectively show the number of cells with phenotype A and B at each point of the medium. In our simulations, we used $D_s = 10^{-10} m^2/s$, $k_{sp} = 0.01 kg^{-1} s^{-1}$, $k_{sc} = 10^{-3} kg^{-1} s^{-1}$, $k_{sd} = 10^{-2} s^{-1}$, and $K_s = 0.01 m^{-2}$. In these simulations, the secreting cells are those with phenotype B; hence, the production of signaling molecules is proportional to the amount of B cells. Since both phenotypes consume these molecules, the consumption depends on the number of both A and B cells. When B cells emerge, they secret signaling molecules, which diffuse in their environment and lessen the production of protein X and augment the production of protein Y. Consequently, their surrounding cells would have less chance of producing protein X and their offspring is less likely to be in the domain of attraction of protein X (figure 3).

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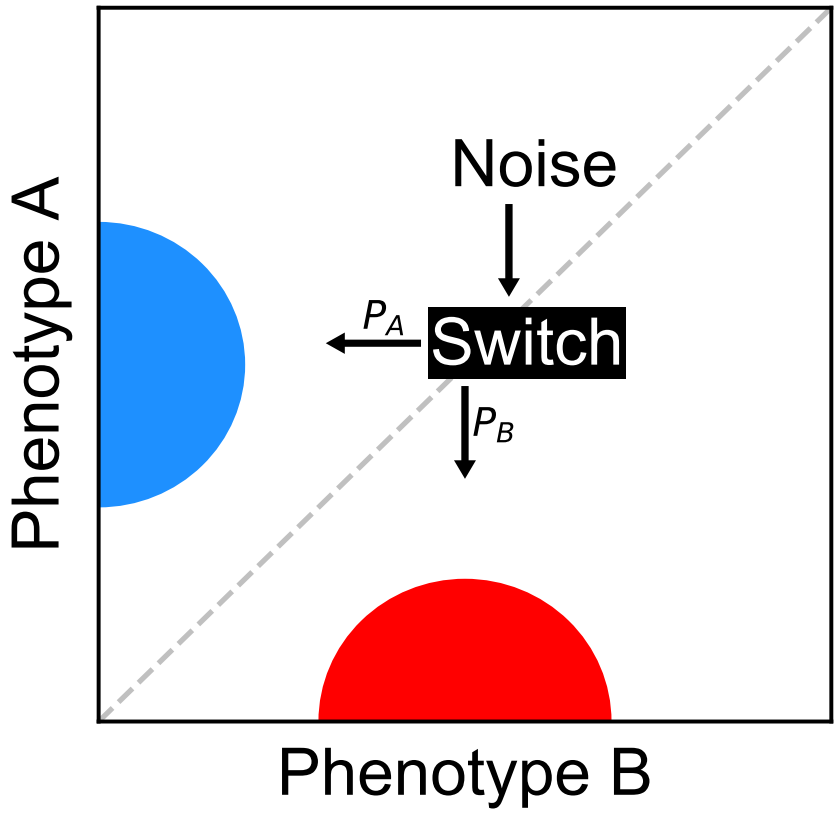


Figure 1. A schematic representation of the NDD model. In a bistable switch, two states are available: A and B. The switch chooses each of these states with probabilities, P_A and P_B, accordingly. The likelihood of a switch choosing one state over the other depends on the noise in its environment as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

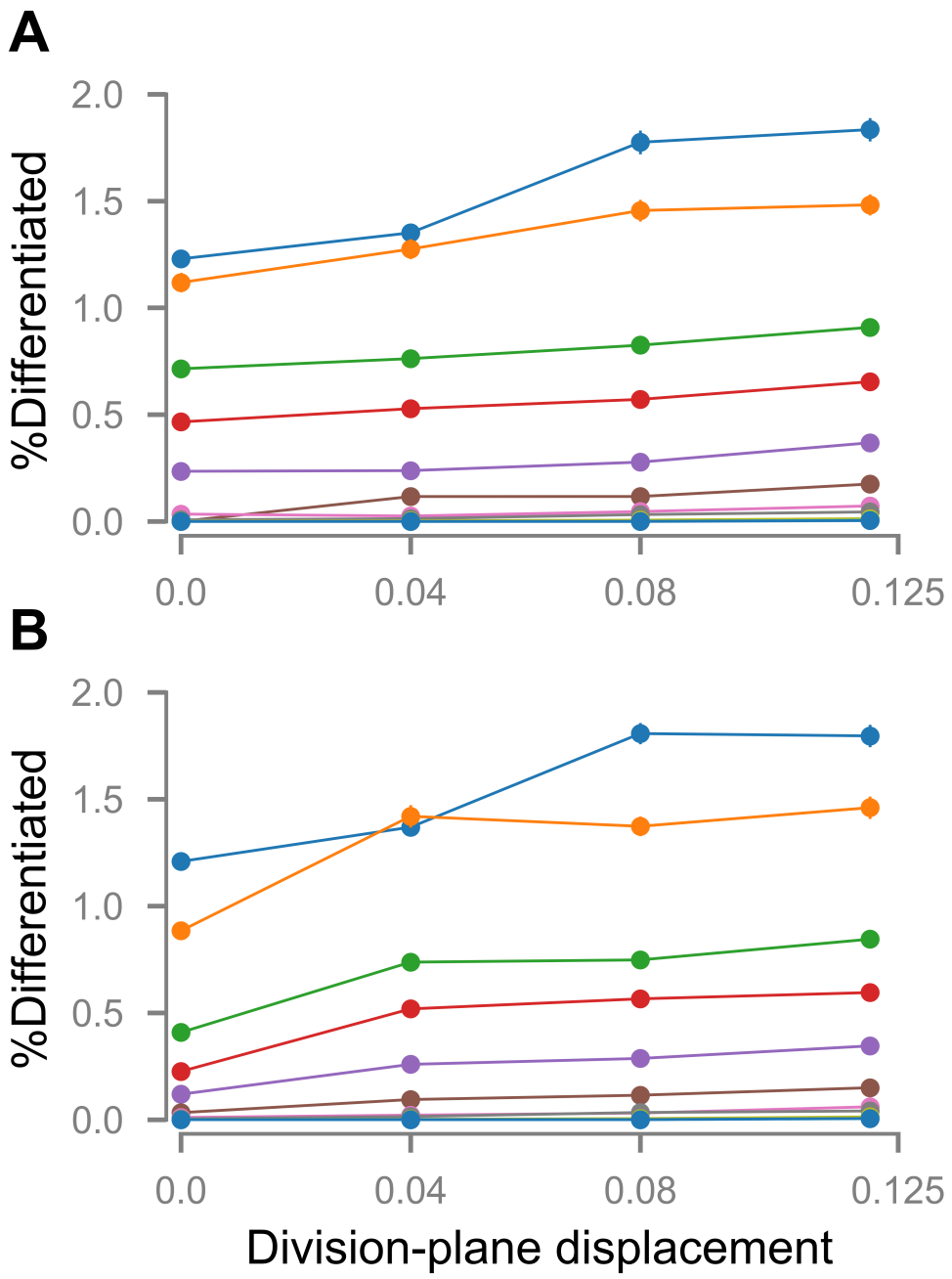


Figure 2. The stochastic positioning of the division plane and the random distribution of TFs in the cytoplasm, as intrinsic sources of noise, affect the none-genetic phenotypic diversity (component #3). The phenotypic diversity is represented by the proportion of cells with the phenotype B relative 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 replications. The parameters used to generate this and the following figure are as follows: $n = 2$, $\beta = 0.1$, protein degradation rate $= 10\text{min}$, and protein dissociation constant $= 10$.

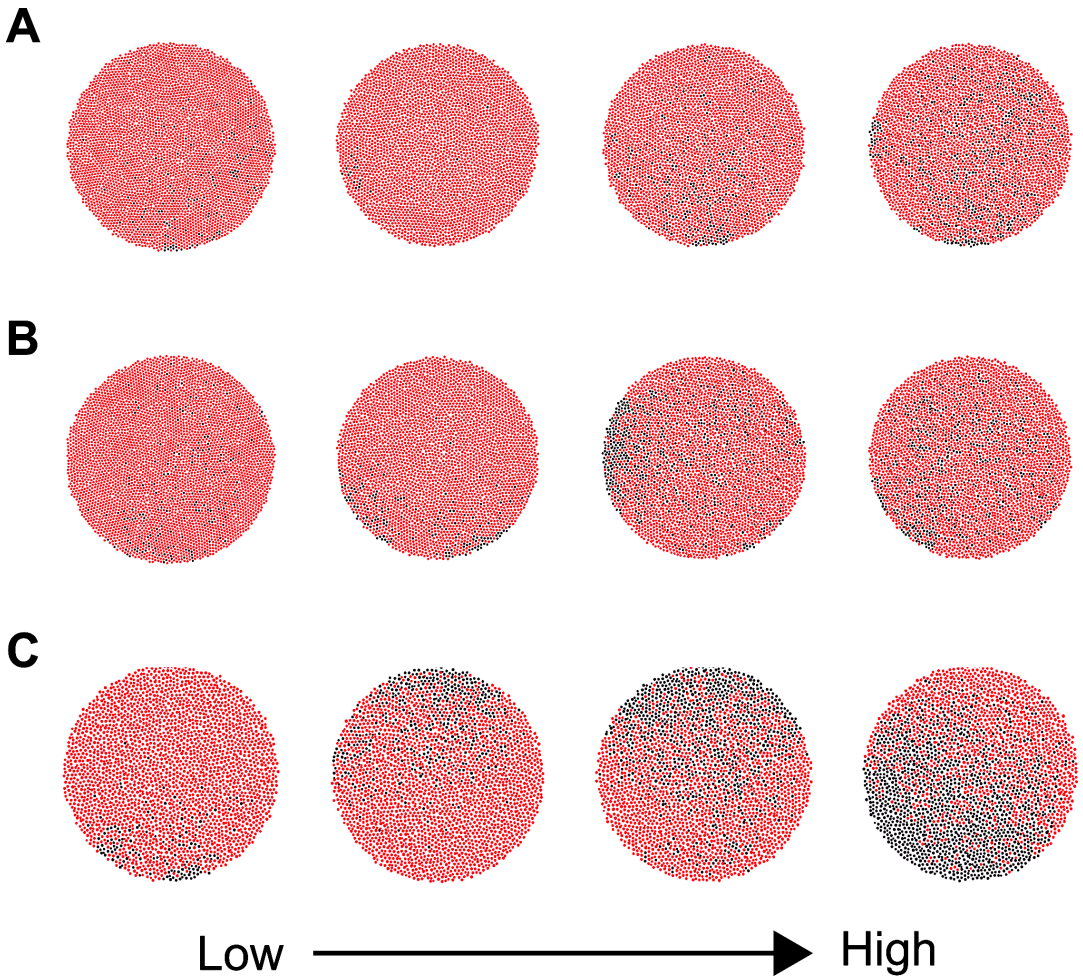


Figure 3. Population heterogeneity as a result of the noise in: (A) the number of TFs in the daughter cells, (B) the position of the division plane, or (C) the secretion of signals from the B cells. The red dots represent the A cells and the black ones represent the B cells. Each circle is the final state of a single run of the 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 a cell is 25 and the radius of the area is $30\mu m$.

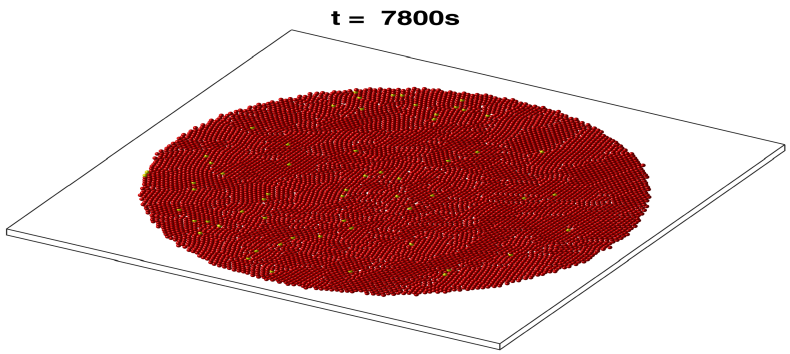
Appendix 1

Movies

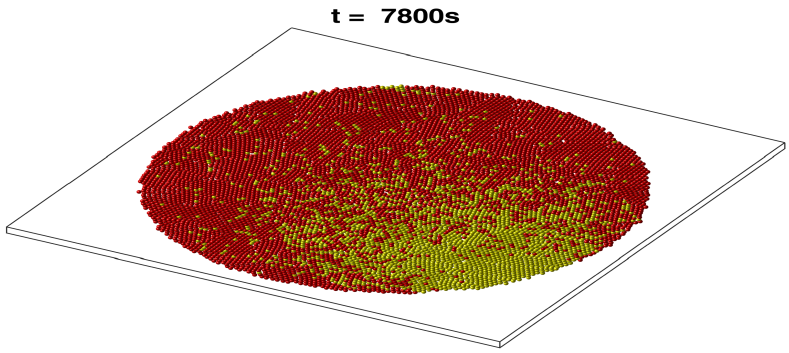
The movies S1 and S2 show a three dimensional simulation of the community of cells in a layer. Simulation performed in a cylinder of radius r and height h and starts with one cell at the center. The cells grow in volume; after reaching a critical volume they divide and the same as two dimensional case, their cytoplasmic content distributes between the two daughter cells.

Movie S1 (`movieS1.avi`): The emergence of heterogeneity in the population of cells as a result of the presence of noise in the process of cell growth and division. The average amount of TFs in each cell at steady state is 25. The simulation started by one cell and continues over 13 generations, $r = 50\mu m$ and $h = 52nm$. Since there is a single layer of cells, h corresponds to the diameter of a single cell.

Movie S2 (`movieS2.avi`): The formation of a spatial organization as a result of the secretion of signaling molecules, which diffuse in their environment and affect the differentiation of the cells. The average amount of TFs in each cell at steady state $= 25$. The simulation started by one cell and continues over 13 generations, $r = 50\mu m$ and $h = 52nm$.



Appendix 1 Figure 1. The final frame of Movie S1



Appendix 1 Figure 2. The final frame of Movie S2