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 cell aggregation. The behavior of this model of cell aggregation is in concert with the NDD model.

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 [1].

A different view has recently gained some grounds [2–5]. 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 [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 ordered spatiotemporal patterns in spatially-extended cell 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, e.g., see [8, 9] as examples of an impressive body of work produced by Kunihiko Kaneko and his colleagues on this subject and [10, 11] as similar proposals regarding the possible role of stochasticity in generating phenotypic diversity. We argue that our approach differs from theirs and similar ideas in certain important aspects: firstly, our model assumes that cell fate is determined when the cell is born, and secondly, that stochastic fluctuations in the cell, and the effect of signals from neighboring cell in the multicellular case, drive the phenotype of the cell towards one attractor rather than another during cell division. This approach is in keeping with the recent emphasis on the importance and the prevalence of noise in biological functions, specifically cell fate [2, 4, 12].

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 [13–15]. Single-cell level measurements of gene expression further cements the notion that cells are intrinsically noisy when it comes translating its genotype into phenotype [16]. The effect of noisy expression of genes on phenotypic diversity in genetically identical cell has been throughly demonstrated in different organisms, from influencing fitness in *Bacillus subtilis* [17] to determining cell fate in mice [18]. (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 [19]) and the second category relies on asymmetric cell division (reviewed in [20]). 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 [21, 22]. 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, this is merely a pedagogical simplification. The position of the plane along which a cell divides is expected to follow a range possibilities, which in its most extreme results in the formation of polar bodies (reviewed in [23–27]). 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, even if molecules are homogeneously distributed within the cell. In fact, the central role of asymmetric cell division in the diversification of cells, from *Drosophila* to mammals has been known for many years [28, 29]. In addition to the noise generated by the stochastic positioning of the division plane, the random distribution of molecules within a 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 [30–33]. 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 idea that characteristics of a cell can be changed by a switch is not novel (e.g., [34]). The notion that cell fate is determined by a switch is best illustrated by the now famous case of the λ phage. 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 [35]. The intrinsic noise in the cell (component #1) can influence the decision made by the toggle switch [36]. Similar switches are not limited to simpler organisms, but can be found in a variety of taxa (reviewed in [2]). (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. In reality, the strength of interactions within the switch make one outcome more likely than the other at a given point in time. For example, upon infecting bacterial cells, λ phage proceeds to lyse the host, but as the concentration of CII protein increases, so does the likelihood of the reactions suppressing the activation of pR and pL promoters, relevant to the onset of the lytic trajectory, which in turn, tilts the scale away from lysis towards lysogeny [37]. (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 [38]. Sharifi-Zarchi [39] 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, They 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. 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.** As described in 2 and demonstrated by phenomena such as phenotypic diversification in human embryonic retinoblast cell line [40], we propose that phenotypic diversity arises from the effect of the noise on a genetic circuit that exhibits a switch-like behavior. While stochasticity is what drives the decision made by the switch, i.e., the fate of the cell, the information necessary to construct the switch is encoded in the genetic content of a cell. Our model presumes that different phenotypes are produced from the same genotype as a consequence of noise, a phenomenon widely observed in nature (reviewed in [41]) (component #7)

The components #1-7 are sufficient to generate a population of cells with different proportions of two phenotypes (Fig 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 ordered spatiotemporal patterns in the body of a multicellular organism.

8. **Cell fate is determined by its location and its environment.** 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 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 germ-line cells floating within this cellular sphere [42]. 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)

To test the general veracity of the NDD model, we used a simple model of cell aggregation.

Results

The overall behavior of the cell aggregation model demonstrates the principles of our framework – that is, the stochasticity results in phenotypic heterogeneity as the population grows in size (movie S1). 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 Fig 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 Fig 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 (Fig 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 (Fig 2B).

Signal from other cells can created spatial order

In the cell aggregation 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 population affects the number of proteins involved in the switch – that is, switching to the phenotype B during cell division becomes more likely. When this environmental signaling is added to the population, the cells organize in a non-random fashion, a stark contrast to the random heterogeneity observed before (Movie S1).

Fig 3 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 (Fig 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, evident by an increase in the clustering coefficient (defined as $\frac{\#\text{triangle loops}}{\#\text{connected triples}}$) for B cells (0.4484 - 0.4787 - 0.5078 - 0.9940) (Fig 3C). 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 [43] 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 [44]. Without tangible levels of conflict, multicellularity as a trait becomes patently advantageous. In their seminal work, Maynard Smith and Szathmáry [45] 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. It is tempting to postulate a connection between the cell-differentiation switch, postulated in the NDD model, and the toggle switch used in quorum sensing in bacteria [46]. Quorum sensing enables bacteria to regulate their phenotypes apropos of their neighbors and is more robust in a dense community [47]. It seems plausible to consider this type community-based phenotypic regulation as a precursor to similar switch-based mechanisms for cell differentiation in multicellular organisms.

In their criticism of a noise-driven alternative to their model, Suzuki [8] considered it unlikely for a noise-driven model to maintain the exact levels of stochasticity needed to produce the desired proportion of differentiated cells to stem cells. In our view, this 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 in the genes involved with the functionality of the switch ($\frac{dN}{dS}$), we would expect $\frac{dN}{dS} < 1$, which signifies the effect of negative selection on the switch [48]. Suzuki [8] 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 indeed reversible, 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, as it should increase the strength of attractors [49].

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 [18, 50]). 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.

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. Our model is the logical extension of earlier ideas describing the role of stochasticity in phenotypic variation and the switch-like behavior of genetic circuits vis-à-vis differentiation and multicellularity (e.g., see [51]).The model of cell aggregation used in this study allowed us to test all the components of the NDD model, barring components 6 and 7, which demand through investigations of their own. This model of cell aggregation provides us with a relatively realistic depiction of the process that results in phenotypic differentiation in a population. We believe that, with few changes, the NDD model can be applied to other biological systems as well.

Materials and methods

In the cell agregation model, the population 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 model, a cell can have two phenotypes, A and B, as shown in Fig 1. The dominance of protein X leads to phenotype A and the dominance of protein Y results in phenotype B. In fact, a positive feedback loop influences the 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 [52]. 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 [52–54]. Biologically-reasonable values were chosen for the parameters used in our simulation such that Eq 1 would be bi-stable (following [52]). 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, which determines its fate. Depending on the intensity of inhibitory effects of TFs (through the values of constants in the Hill function [52]), the two domains of attractors could be equal or not (component #5).

Population growth algorithm

Simulation starts with a single cell with phenotype A. Each iteration in the simulation can be divided into four steps:

1. *Cell growth*: In this step, 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 [55] 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. In reality and in the presence of active transportation, one can still expect a uniform distribution of molecules in the cytoplasm [32], making this assumption biologically reasonable. 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. Since in our simulations, daughter cells have similar volumes, we consider the number of proteins distributed between them, and not their concentrations.

3. *Relaxation*: After a cell divides, the cells push each other outwards to make room for the new daughter cells [56]. 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 $D_s = 10^{-11} m^2/s$, $k_{sp} = 0.01 kg^{-1} s^{-1}$, $k_{sc} = 0.0001 kg^{-1} s^{-1}$, $k_{sd} = 0.01 s^{-1}$, and $K_s = 0.01 m^{-3}$.

   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. The minimum effective concentration of the signaling molecules at any location determines if a cell at that location is affected by the signal, which would decrease 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.

Code availability

The software used to run all simulations was Matlab 2016 and the scripts are available at `https://github.com/hasafdari/Noise_Driven_Cell_Differentiation` (doi: `https://doi.org/10.5281/zenodo.1227287`).

Supporting information

SI Movies

Movie S1 and Movie S2 show 3-dimensional simulations of a 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.

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Additional information

The authors declare no competing interests.

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Author contribution

MS designed research; RT and BG 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.

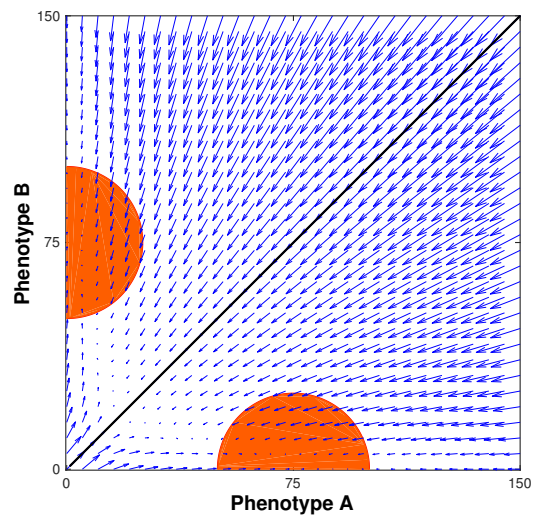

Fig 1. The phase-portrait diagram for the NDD model (based on Eq 1). In a bistable switch, two states are available: A and B. The switch chooses each of these states with probabilities, P_A and P_B, accordingly. The likelihood of a switch choosing one state over the other depends on the noise in its 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, The parameters used to generate this and the following figure are as follows: $n = 2$, $\beta = 0.1$, protein half-life = 10min, and protein dissociation constant = 10. Unless noted otherwise, these parameters are used in all the subsequent figures.

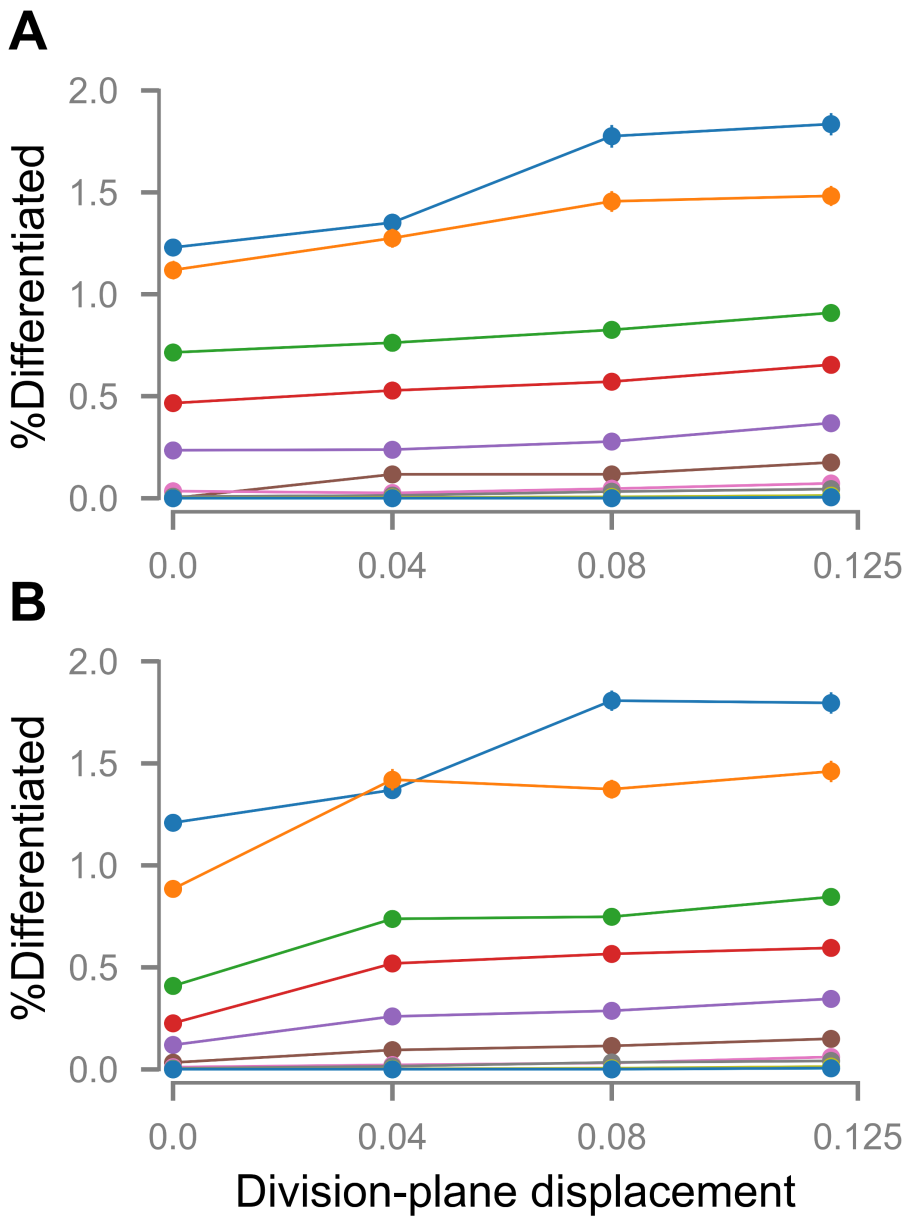

Fig 2. The stochastic positioning of the division plane and the random distribution of TFs in the cytoplasm, as intrinsic sources of noise, affect the none-genetic phenotypic diversity (component #3). The phenotypic diversity is represented by the proportion of cells with the phenotype B relative to 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. Error bars are 95%CI.

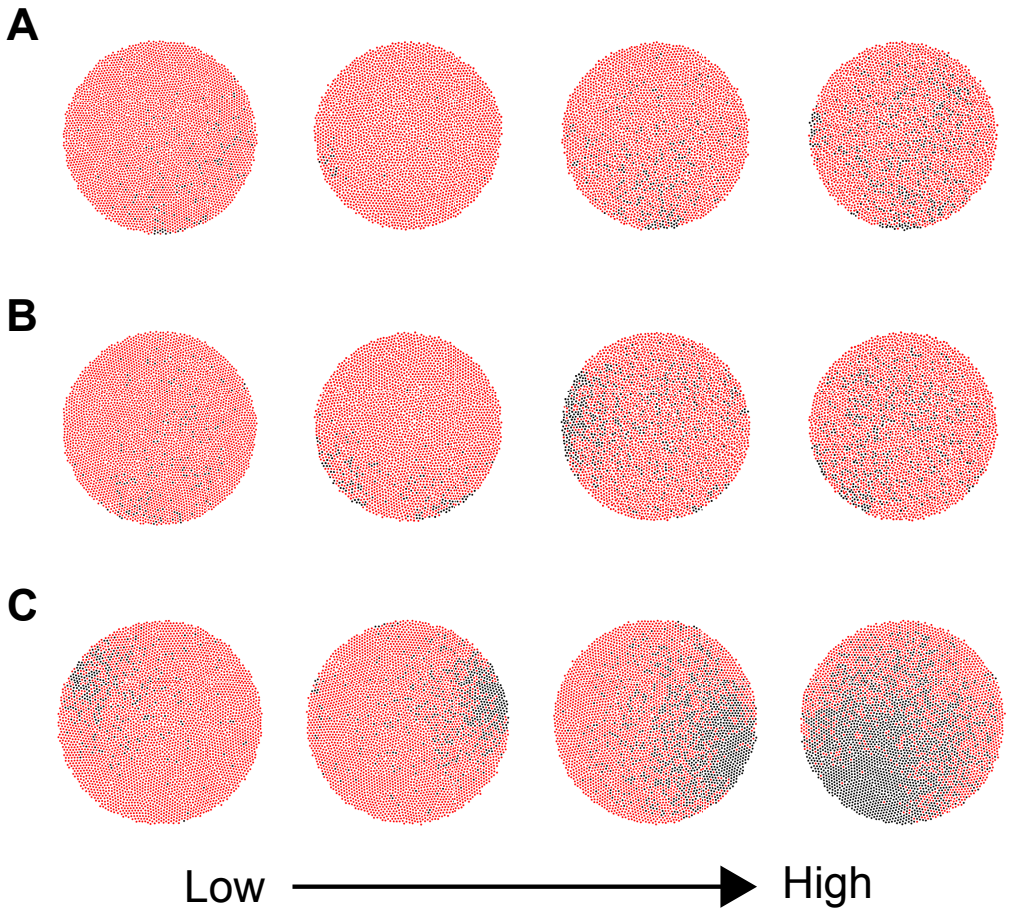

Fig 3. Population heterogeneity as a result of the noise in: (A) the number of TFs in the daughter cells, (B) the position of the division plane, or (C) the secretion of signals from the B cells. The red dots represent the A cells and the black ones represent the B cells. Each circle is the final state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

Movie S1 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 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 $h = 52nm$.