Mechanisms of Formation of Biological Signaling Profiles

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Abstract.

Formation and growth of multi-cellular organisms and tissues from several genetically identical embryo cells is one of the most fundamental natural phenomena. These processes are stimulated and governed by multiple biological signaling molecules, which are also called morphogens. Embryo cells are able to read and pass the genetic information by measuring the non-uniform concentration profiles of signaling molecules. It is widely believed that the establishment of concentration profiles of morphogens, commonly referred to as morphogen gradients, is a result of complex biophysical and biochemical processes that might involve diffusion and degradation of locally produced signaling molecules. In this review we discuss various theoretical aspects of the mechanisms for morphogen gradients formation, including stationary and transient dynamics, the effect of source delocalization, diffusion, different degradation mechanisms, and the role of spatial dimensions. Theoretical predictions are compared with experimental observations. In addition, we analyze potential alternative mechanisms of delivery of biological signals in embryo cells and tissues. Current challenges in understanding the mechanisms of morphogen gradients and future directions are also discussed.

PACS numbers:

1. Introduction

Development of various living organisms from initially a very small group of identical embryo cells is one of the most fascinating and complex processes in biology [1–4]. A critical stage in biological development is a pattern formation during which the eventual fates of cells become determined at different times and different spatial positions. Several classes of biological signaling molecules, known as morphogens, play a central role in the tissue patterning and organ formation in all living systems [1–5]. The term 'morphogen' was first introduced by A. Turing in his seminal paper on mathematical modeling of biological pattern formation [6]. In this pioneering work, Turing demonstrated that diffusion and chemical reactions involving several species can produce spatial patterns in an array of cells, which are very similar to patterns observed in nature. Recently experiments provided a direct verification for Turing's theory in artificial chemical systems [61]. The next critical step was made by L. Wolpert who introduced the idea of positional information [4, 13]. He argued that the developmental pattern formation is a result of interpretation of spatial positions decoded in external signals from biological signaling molecules. Cells obtain the spatial information by somehow "measuring" the concentration of morphogens around them. Different genes are turned on or off depending on several concentration thresholds, producing eventually morphologically different cells. Finally, F. Crick realized that dynamic aspects of the creation of morphogen profiles are also important for proper development of tissues and organs [5]. He emphasized the important role of the diffusion because of limited time window for development processes. These three fundamental concepts are the foundations of modern theory of morphogen gradients [7–10, 12].

Enlightened by these ideas, a large number of experiments on formation, regulation and functioning of morphogen gradients has appeared in recent years. They clearly show that the non-uniform concentration profiles of signaling molecules are responsible for symmetry breaking, tissue development and organs formation in multi-cellular organisms [7–10, 12, 14–19, 24–27, 29, 33, 41, 46, 83]. Although these investigations of morphogen gradients were done on diverse biological systems, most studies focused on two main examples. The first one is a Drosophila embryo where Bicoid (Bcd) morphogen gradients and the corresponding patterns were monitored and analyzed [7–9, 16, 27, 33, 46]. The second example is a pattern formation in vertebrate neural tubes by Sonic hedgehog (Shh) morphogens [8, 20, 48, 49]. A large amount of quantitative information has been assembled on these systems, which highlighted the importance of morphogen gradients. Another striking observation is that, despite different evolutionary origins, distinct cell biology and biochemistry, the formation of tissue patterns is very similar in both organisms [8].

Analysis of developmental processes in different systems suggested the existence of several universal mechanisms governing the establishment of signaling profiles and their activities [7–9, 12]. Stimulated by these experimental observations, a large number of theoretical ideas on the mechanisms of formation of morphogen gradients have been proposed [7–10, 12, 21, 21–23, 31, 32, 34–40, 43, 50, 51, 58, 59, 76–78, 80]. Most of these studies suggest that the establishment of biological signaling profiles in development is a result of complex physical-chemical processes that include the localized production of morphogens that later can diffuse and be removed from the cellular medium by various types of biochemical transitions [7–10, 12]. Based on some experimental observations [64,65,67,68,70,71,74], the possibility of alternative mechanisms of the direct delivery of morphogens to the target cells utilizing dynamic cellular extensions called cytonemes was predicted [11, 64, 77]. It has been argued that the complex environment of the embryo systems might prevent the free diffusion from establishing the distinguishable morphogen gradients at different regions, implying a different mechanism of the biological signal transduction [11, 64].

In this paper, a brief overview on the mechanisms of development of morphogen gradients is presented. Because of the large number of excellent reviews on morphogens [7–10, 12], we concentrate here only on the theoretical aspects of the formation of biological signaling profiles. For this reason, the important subjects such as how cells interpretate the graded signals and biochemical regulations of these signals are not discussed [12, 47]. Furthermore, in analyzing the complex processes associated with the establishment of morphogen gradients we benefited from multiple discussions with many researchers. But this review presents our subjective view on the field, which might disagree with some existing descriptions.

2. Development of Morphogen Gradients via Reaction-Diffusion Processes

The dominating view in the field is that the morphogen gradients are created by a complex action of several reaction-diffusion processes [7–10, 12]. Two main theoretical directions have been explored in clarifying the mechanisms of formation of signaling profiles. One of them utilizes a continuum description, while another one is based on more general discrete-state stochastic analysis of the processes. Below we discuss and compare both of them.

2.1. Continuum Synthesis-Diffusion-Degradation Model

Experiments show that biological signaling profiles are not uniform as shown in Fig. 1. This lead to the formulation of the simplest and still the most popular idea on the formation of morphogen gradients. It is known as a *Synthesis-Diffusion-Degradation* (SDD) model [7–10, 12]. It is the most frequently applied model for analyzing multiple systems where the profiles of signaling molecules are formed [7–10, 12].

To describe this model mathematically, let us consider a semi-infinite signaling domain in which morphogens are produced at the origin, $x = 0$, with a rate Q. They diffuse along the field of cells with a diffusion constant D or they might be degraded with a rate k. One can define a function $C(x, t)$ as concentration of morphogens at position x at time t. The temporal evolution of the concentration profile follows from a

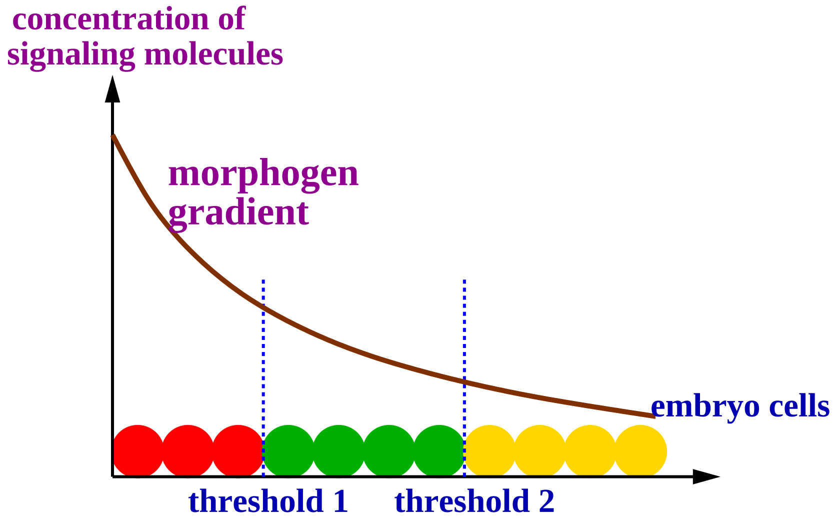


Figure 1. A schematic view of the morphogen gradient and its action on embryo cells. Cells exposed to the morphogen concentration above the threshold 1 will activate a "red" gene; cells exposed to the morphogen concentration below the threshold 1 but above the threshold 2 will activate a "green" gene; and cells exposed to the morphogen concentration below the threshold 2 will activate a "yellow" gene.

corresponding reaction-diffusion equation,

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2} - kC(x,t), \tag{1}$$

with the boundary condition at the origin

$$\frac{\partial C(x,t)}{\partial x}|_{x=0} = -Q. \tag{2}$$

Assuming that initially there were no morphogens in the system, $C(x,t=0)=0$, these equations can be exactly solved at all times, yielding the following concentration profile [23],

$$C(x,t) = C^{(s)}(x)\left[1 - \frac{1}{2}erfc\left(\frac{\sqrt{Dt}}{\lambda} - \frac{x}{2\sqrt{Dt}}\right) - \frac{1}{2}e^{-2x/\lambda}erfc\left(\frac{\sqrt{Dt}}{\lambda} + \frac{x}{2\sqrt{Dt}}\right)\right] \tag{3}$$

where $erfc(y)$ is the complementary error function, $\lambda = \sqrt{D/k}$, and $C^{(s)}(x)$ is the stationary profile,

$$C^{(s)}(x) = \frac{Q}{\sqrt{Dk}}\exp\left(-\frac{x}{\lambda}\right). \tag{4}$$

The SDD model predicts that at large times ($t \to \infty$) the morphogen gradient is the exponentially decaying function of the distance from the source with a length scale λ determined by the ratio of the diffusion and degradation rates. These predictions qualitatively agree with many observations of morphogen gradients in various systems [16, 19, 24, 27, 46]. However, the application of this model for more quantitative measurements of the dynamics of formation of signaling profiles in Bcd led to some controversial results [16]. Experiments suggested that Bcd molecules diffuse relatively slow with $D \simeq 0.3\ \mu\mathrm{m}^2/\mathrm{s}$ [16]. The most distant location that is still affected by the bicoid molecules is at $L \simeq 375\ \mu\mathrm{m}$ [34]. Using the unbiased random walk arguments, one can evaluate how long it takes for the morphogens to diffuse to this location, $\tau = L^2/2D \simeq 4000$ minutes, providing the estimate for the formation of Bcd profile in drosophila embryos. But this contradicts to the experimental observation that the morphogen gradient is created in approximately 90 minutes in the whole embryo system. Clearly, there is a discrepancy between these theoretical predictions and what is measured directly in experiments [16]. Several early attempts to explain this controversy turned out to be unsuccessful [79].

A progress in resolving this paradox of slow diffusion and fast formation of the morphogen gradients has been achieved by Berezhkovskii and coworkers who introduced a new method of analyzing the dynamics [21, 34–36, 43]. They realized that the correct estimate of the times to establish the morphogen gradient is given by relaxation times to reach stationary-state profiles, which are labeled as *local accumulation times* (LAT) [34–36]. To obtain LAT one has to define first local relaxation functions,

$$R(x,t) = \frac{C(x,t) - C^{(s)}(x)}{C(x,t=0) - C^{(s)}(x)} = 1 - \frac{C(x,t)}{C^{(s)}(x)}. \tag{5}$$

The physical meaning of these functions is that they represent a relative distance to the stationary state: at $t = 0$ the distance is one, while at the steady state it is equal to zero. The function $\left(-\frac{\partial R(x,t)}{\partial t}\right)$ is the probability density for reaching the stationary state at the position x at time t. The explicit formulas for the local accumulation time can be derived then via Laplace transformations of the local relaxation function, $\widetilde{R}(x,s) = \int_0^\infty R(x,t)e^{-st}dt$ [34],

$$t(x) = \int_0^\infty t\left(-\frac{\partial R(x,t)}{\partial t}\right)dt = \widetilde{R}(x, s=0). \tag{6}$$

For the SDD model, from Eqs. (3) and (5) it can be shown that the relaxation function is given by

$$R(x,t) = erfc\left(\frac{\sqrt{Dt}}{\lambda} - \frac{x}{2\sqrt{Dt}}\right) + \frac{1}{2}e^{-2x/\lambda}erfc\left(\frac{\sqrt{Dt}}{\lambda} + \frac{x}{2\sqrt{Dt}}\right), \tag{7}$$

which leads to a very simple expression for the LAT [34],

$$t(x) = \frac{1}{2k}\left(1 + \frac{x}{\lambda}\right). \tag{8}$$

For Bcd morphogen gradient, using the expression (8) along with the estimate of the decay length $\lambda \simeq 60\ \mu\mathrm{m}$ and with a better estimation of the diffusion constant $D \simeq 1\ \mu\mathrm{m}^2/\mathrm{s}$ [22, 53], the time to reach the stationary state at the most distant boundary was calculated to be less than 200 minutes, which is much closer (although still not perfect) to the experimental values ($\simeq 90$ minutes). The difference between these theoretical predictions and experiments is probably due to not precise measurements of the diffusion constant and the decay length, as well as due to oversimplified theoretical assumptions of the strongly localized source region, as we discuss below in more detail [34]. Thus, the systematic approach to evaluate LAT as a measure of the dynamics of the formation of morphogen gradients was able to mostly resolve the paradox of slow diffusion [34]. However, it also raised several fundamental questions. Eq. (8) indicates a linear scaling as the distance from the source for the SDD model instead of the expected quadratic scaling for the unbiased random walk motion since there are no apparent external driving forces in the system. It led to a conclusion that signaling profiles formed much faster than was previously estimated [34, 45, 57]. But the mechanism of this acceleration was not clear.

2.2. Discrete-State Stochastic Description

During the establishment of the signaling profile, the morphogen molecules are removed from the medium at specific locations of the cells (usually at receptors), and this implies that the overall process is intrinsically biochemically discreet. This suggests that the continuum description of the formation of morphogen gradients is an approximation, and it might not fully describe these processes at all conditions [57]. For this reason, a more general discrete-state stochastic framework was introduced [57].

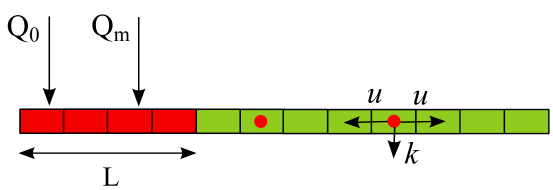


Figure 2. A schematic view of the one-dimensional discrete-state SDD model for the formation of the morphogen gradients. The production of morphogens is distributed over an interval of length L. Signaling molecule are produced at the sites $0 \leq m \leq L$ (shown in red) with rates Q_m. The case of $m = 0$ and $L = 1$ corresponds to the source localized at the origin. Particles can also diffuse along the lattice to the neighboring sites with a rate u, or they might be degraded with a rate k. Adapted with permission from Ref. [75].

The discrete version of the SDD model is presented in Fig. 2. It is assumed that each embryo cell is associated with a lattice site. In the simplest version of the model, the morphogens are produced only at the origin with a rate $Q_0 = Q$ ($L = 1$ case in Fig. 2). Signaling molecules can diffuse with a rate u along the lattice. At any position, the morphogen can be degraded and removed from the system with a rate k. The continuum description is obtained in the limit of very fast diffusion, $u \gg k$. To simplify calculations, a single-molecule view, according to which the concentration of morphogens at given site is equivalent to a probability to find the signaling molecule at this location, was adopted [57]. A function $P_n(t)$, defined as the probability to find the morphogen at site n at time t, was introduced [57]. The temporal evolution of this probability is governed by the following master equations,

$$\frac{dP_0(t)}{dt} = Q + uP_1(t) - (u + k)P_0(t) \tag{9}$$

for $n = 0$; and

$$\frac{dP_n(t)}{dt} = u[P_{n-1}(t) + P_{n+1}(t)] - (2u + k)P_n(t) \tag{10}$$

for $n > 0$. At large times, where $\frac{dP_n(t)}{dt} = 0$, these equations can be easily solved, producing the exponentially decaying profile,

$$P_n^{(s)} = \frac{2Qx^n}{k + \sqrt{k^2 + 4uk}} \tag{11}$$

with

$$x = (2u + k - \sqrt{k^2 + 4uk})/(2u). \tag{12}$$

In the continuum limit, $u \gg k$, Eq.(11) reduces, as expected, to the expression (4). The characteristic length of the concentration decay is given by

$$\lambda = -\frac{1}{\ln x}. \tag{13}$$

In the case of fast diffusion rates, $u \gg k$, this length is equal to $\lambda \simeq \sqrt{u/k}$ which is a well-known result for the continuum SDD model. [34] In another limit of fast degradation rates, $k \gg u$, this length is very small $\lambda \simeq \frac{1}{\ln(k/u)}$ because the morphogen molecules cannot move large distances from the source due to fast degradations [57].

Calculating LAT for the discrete-state version of the SDD model, the linear scaling was found again [57],

$$t_n = \frac{1}{\sqrt{k^2 + 4uk}} \left[\frac{2u + k + \sqrt{k^2 + 4uk}}{k + \sqrt{k^2 + 4uk}} + n \right]. \tag{14}$$

In the limit of fast diffusion rates, which describes the continuum regime, this expression reduces to Eq. (8), while at another limit of fast degradation rates it produces $t_n \simeq (n+1)/k$. But for all regimes a linear scaling as a function of the cell position n is again observed, implying faster than expected the formation of the morphogen gradient.

To explain such fast relaxation to the stationary-state profiles, the following arguments were presented [57]. Initially, at $t = 0$, the morphogen molecules start at the origin ($n = 0$), and there is nothing at the site $n > 0$. Then the relaxation time to reach the stationary-state at the site n should be consisting of two contributions. The first one comes from the fact that the signaling molecules first have to reach the site n, and it can be associate with a mean first-passage time (MFPT) to arrive here. It is expected that MFPT should strongly depend on n. The second contribution is due to local fluctuations at the given site until the stationary-state conditions are reached. It was argued that this term, labeled as a *local rearrangement time*, is weakly dependent on the position along the lattice. Thus, at large distances from the origin ($n \gg 1$) the local relaxation time can well approximated by the MFPT, which can be calculated exactly using the backward-master equations [57, 81, 82]. These arguments are illustrated in Fig. 3 where the ratios of LAT to MFPT are plotted as a function of the distance from the source. In all cases, both time scales approach to each other at large n. Later these theoretical predictions were explicitly proven by Berezhkovskii and Shvartsman [35], who also showed that the local rearrangement time can be viewed as the LAT if the source is localized at the observation point.

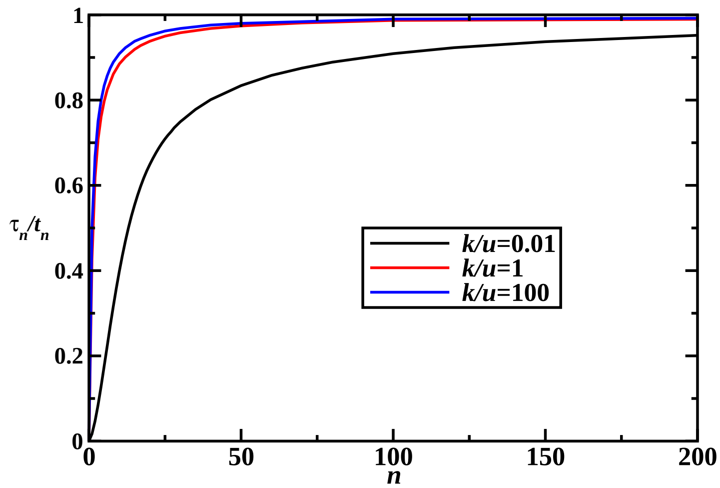


Figure 3. Ratio of LAT and MFPT for one-dimensional discrete-state stochastic SDD model as a function of the distance from the source for different diffusion and degradation rates. Reprinted with permission from Ref. [57]. Copyright 2011 American Chemical Society.

But the fact that the LAT can be well approximated by the first arrival time is not enough to explain the fast relaxation to the stationary-state profiles. It was argued that the critical part of the process is the particle removal from the medium [57]. Morphogen molecules have a nonzero probability to be removed from the system at each site due to degradation. To survive on the lattice, the morphogen molecules must move faster or they will be removed. This corresponds to effective speeding-up of the surviving signaling molecules. It can be also viewed as a fact that the degradation creates an effective potential $U_{eff}(n)$ that drives the morphogens along the lattice away from the source. This potential can be estimated from the stationary-state profile [57],

$$U_{eff} \simeq k_B T \ln P_n^{(s)}. \tag{15}$$

For the discrete-state SDD model this leads to the strongly decreasing linear potential, $U_{eff} \simeq n \ln x = -n/\lambda$, which implies that there is a constant effective force,

$$F_{eff} = -\frac{\partial U_{eff}(n)}{\partial n} = \frac{1}{\lambda}, \tag{16}$$

which drives the morphogens away from the origin. However, the most important conclusion from these arguments is that the motion of signaling molecules in such potential is a driven process. It is not the unbiased diffusion as was earlier assumed. This should naturally lead to the linear scaling in times [57]. It is important to note that each molecule has no bias in its motion, but because the concentration due to the degradation decreases for larger n the overall flux in the system will be flowing in the direction of larger n. This is similar to the effect of the constant force acting in the positive direction. These theoretical arguments provide a microscopic explanation for the fast formation of morphogen gradients, and they also emphasize the critical role of the degradation processes [57].

2.3. The Effect of Source Delocalization

Although the simplest SDD model was able to explain some aspects of the development of morphogen gradients, it was pointed out that many realistic features of the process that might strongly influence the dynamics are not taken into account [30, 31, 34–37, 57, 75]. Experiments show that in many biological systems the production region of signaling molecules is not strongly localized as assumed in the simplest SDD model [7–10, 12, 30]. Morphogens are protein molecules that must be first synthesized from the corresponding RNA molecules, but the distributions of RNA species in various embryos are more diffuse [42]. For example, for the bicoid system it is known that the maternal RNA molecules can be found in the region of size 30-50 μm, which should be compared with the total length of embryo of $\simeq 400$ μm [7].

To understand the role of spatial delocalization of the production region, several theoretical investigations have been performed [31,37,75]. The formal general solution to describe the formation of morphogen gradients from an arbitrary source (as presented in Fig. 2) has been obtained using the Green's function method in the continuum approximation [31, 37]. In this case, the corresponding reaction-diffusion equation can be written as

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2} - kC(x,t) + S(x,t), \tag{17}$$

where $S(x,t)$ is a function that describes the maternal RNA distribution. It was shown that the time-dependent solution of this equation is given by [31, 37],

$$C(x,t) = \int ds \int dy G(x-y, t-s) S(y,s), \tag{18}$$

where $G(x,t)$ is the Green's function for this system,

$$G(x,t) = \frac{1}{\sqrt{4\pi D t}} \exp\left(-\frac{x^2}{4Dt}\right) \exp\left(-kt\right). \tag{19}$$

The physical meaning of this function is a probability to find the particle at the position x at time t if it started from the origin at $t = 0$ [37].

Using this approach the problems of the formation of morphogen gradients with the source production uniformly distributed over the interval has been analyzed [31,37]. It was found that the stationary-state profile is flattening near the origin, in agreement with observations for Bcd signaling profiles. In addition, it was argued that the almost constant profile at the beginning of the embryo region explains why the target genes are never expressed close to the origin: a sharper gradient is needed in order to reliably turn off genes at the specific locations [31, 37]. Furthermore, using this method more complex normal distribution of production over the whole embryo length as well as time-dependent source productions were investigated. A similar analysis, which emphasized more the diffusion of RNA in the establishment of the signaling profiles, has been done in Ref. [62]. The dynamics of the development of morphogen gradients in the continuum approximation was evaluated using the LAT [37]. Specifically, the case of the exponentially distributed source over the semi-infinite interval, i.e. for $L \to \infty$ in Fig. 2, with the productions rates

$$S(x) = \frac{Q}{\lambda_s} \exp\left(-\frac{x}{\lambda_s}\right) \tag{20}$$

was studied. Here, λ_s is the average decay length for the exponential distribution. It was shown that for this system LAT is equal to [37]

$$\tau_L(x, \lambda_s) = \frac{1}{2k}\left[\left(1+\frac{x}{\lambda}\right)\frac{\lambda e^{-x/\lambda}}{\lambda e^{-x/\lambda} - \lambda_s e^{-x/\lambda_s}} + \frac{2\lambda_s^2}{\lambda_s^2 - \lambda^2}\right]. \tag{21}$$

For the case of $\lambda_s = \lambda$ this equation simplifies into

$$\tau_L(x, \lambda_s = \lambda) = \frac{1}{4k}\left[3 + \frac{x^2}{\lambda(x+\lambda)}\right]. \tag{22}$$

This expression can be used to estimate the times to create Bcd morphogen gradients. Using $\lambda \simeq 60$ μm, $x \simeq 6\lambda$, and $D \simeq 1$ μm^2/s, one can obtain that $\tau \simeq 120$ minutes, which is very close to the experimentally observed 90 minutes [16]. These calculations suggest that the extended source accelerates the dynamics of the development of signaling profiles.

A more general theoretical method to evaluate the role of the source delocalization was introduced later [75]. A discrete-state stochastic SDD model in one dimension with the extended source range, as illustrated in Fig. 2, was considered. It was assumed that the signaling molecules are produced over the interval of length L with rates Q_m for $0 \le m \le L$: see Fig. 2. The total production rates is equal to $Q = \sum_{m=0}^{L} Q_m$. Because the production of morphogens at different sites is independent from each other, it was suggested that the general solution for the probability to find a signaling molecule at the site n at time t with a delocalized production region as specified in Fig. 2, $P(n,t)$, can be written as a sum of the probabilities $P(n,t;m)$ for the single localized sources at the sites m [75]. More specifically, the probability function $P(n,t;m)$ is governed by the following master equations,

$$\frac{dP(n,t;m)}{dt} = Q_m \delta_{m,n} + u[P(n-1,t;m) + P(n+1,t;m)] \\ -(2u+k)P(n,t;m) \tag{23}$$

for $n > 0$, and

$$\frac{dP(0,t;m)}{dt} = Q_0 \delta_{m,0} + uP(1,t;m) - (u+k)P(0,t;m). \tag{24}$$

for $n = 0$. In the steady-state limit, $t \to \infty$, these equations can be solved explicitly, yielding

$$P_1^{(s)}(n;m) = \frac{Q_m[(k+\sqrt{k^2+4uk})x^{m-n} + (-k+\sqrt{k^2+4uk})x^{n+m})]}{(k+\sqrt{k^2+4uk})\sqrt{k^2+4uk}} \tag{25}$$

for $0 \leq n \leq m$, and

$$P_2^{(s)}(n;m) = \frac{Q_m[(k+\sqrt{k^2+4uk})x^{n-m} + (-k+\sqrt{k^2+4uk})x^{n+m})]}{(k+\sqrt{k^2+4uk})\sqrt{k^2+4uk}} \quad (26)$$

for $m \leq n$. The parameter x is given in Eq. (12). Then using the superposition arguments, it can be shown that

$$P(n,t) = \begin{cases} \sum_{m=0}^{n} P_2(n,t;m) + \sum_{m=n+1}^{L} P_1(n,t;m), & \text{for } 0 \leq n \leq L; \\ \sum_{m=0}^{L} P_2(n,t;m), & \text{for } L \leq n. \end{cases} \quad (27)$$

This method allowed to analyze the formation of morphogen gradients for arbitrary length of the production region and for arbitrary production rates [75]. In addition, it also lead to computing the dynamic properties for the development of the signaling profiles by evaluating the relaxation to the stationary-state profiles [75]. To clarify the role of the source delocalization, the development of the morphogen gradients with uniform distributed production over the finite interval and with the exponentially distributed production along the semi-infinite interval were compared with the formation of the signaling profile in the case of sharply localized source at the origin [75]. The corresponding density profiles are presented in Fig. 5, while the estimated LAT are given in Fig. 6. It was concluded that the extended sources delivered the signaling molecules much further in comparison with the single localized source. In addition, the delocalized sources were able to create sharp boundaries which are needed to controllably turning genes on. They were also generally faster in reaching the stationary states.

2.4. The Formation of Morphogen Gradients in Two and Three Dimensions

Most of theoretical models applied for describing the establishment of the biological signaling profiles are essentially one-dimensional [34–37,57,75]. However, a more realistic description of these processes should take into account a complex structure of the embryos [22, 52]. This led to multi-dimensional generalizations of the original SDD models [43, 44, 60].

First, continuum radially symmetric models were considered [43]. It was assumed that the source region is a sphere of radius R around the origin, and that there is no morphogens in the system at $t = 0$. In this case, the concentration profiles are described by [43, 44]

$$\frac{\partial C(r,t)}{\partial t} = D\left[\frac{\partial^2 C(r,t)}{\partial r^2} + \frac{(d-1)}{r}\frac{\partial C(r,t)}{\partial r}\right] - kC(r,t). \quad (28)$$

for a d-dimensional system. Here D is the diffusion constant, Q is the production rate at the boundary of the source region, k is the linear degradation rate and $r \geq R$. The boundary conditions can be written as [43, 44]

$$-D\frac{\partial C}{\partial r}(R,t) = Q. \quad (29)$$

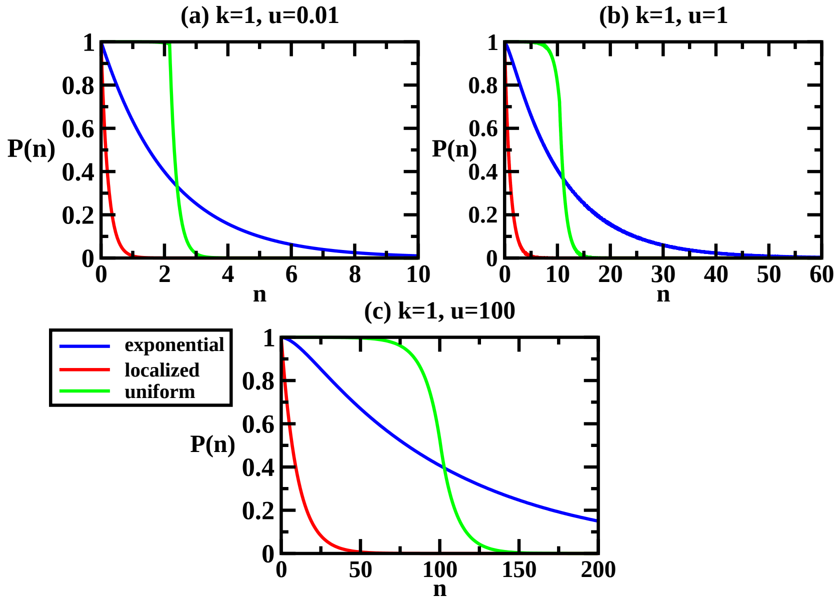


Figure 4. Stationary-state density profiles for the formation of morphogen gradients with different production regions and for variable diffusion and degradation rates. Red curves describe the single-site localized source, green curves describe the uniform production over the finite interval, and blue curves describe the exponential production over the semi-infinite interval. Adapted with permission from Ref. [75].

It was also assumed that far away from the production region the concentration of signaling molecules disappears, $C(r \to \infty, t) = 0$. At large times, the concentration profiles approach the stationary state, which is given by [44]

$$C_{s,d}(r) = \frac{Q}{\sqrt{Dk}} \frac{K_{d/2-1}(r\sqrt{d}/\lambda)}{K_{d/2}(R\sqrt{d}/\lambda)} \left(\frac{r}{R}\right)^{1-d/2}, \tag{30}$$

where $K_m(y)$ is the m-th order modified Bessel function of the second kind and $\lambda = \sqrt{dD/k}$ is the characteristic decay length of the concentration profile in d dimensions. The application of the local relaxation functions provided the explicit expression for times to reach the stationary state [43, 44],

$$\tau_d(r) = \frac{1}{k} - \frac{(R\sqrt{d}/\lambda)}{2D} \frac{K_{d/2+1}(R\sqrt{d}/\lambda)}{K_{d/2}(R\sqrt{d}/\lambda)} + \frac{(r\sqrt{d}/\lambda)}{2D} \frac{K_{d/2}(r\sqrt{d}/\lambda)}{K_{d/2-1}(r\sqrt{d}/\lambda)}. \tag{31}$$

For two dimensional systems ($d = 2$), from Eqs. (30) and (31) one could derive the concentration profile [44],

$$C_{s,d=2}(r) = \frac{Q}{\sqrt{Dk}} \frac{K_0(r\sqrt{2}/\lambda)}{K_1(R\sqrt{2}/\lambda)}, \tag{32}$$

and the LAT,

$$\tau_{d=2}(r) = \frac{(r\sqrt{2}/\lambda)}{2k} \frac{K_1(r\sqrt{2}/\lambda)}{K_0(r\sqrt{2}/\lambda)} - \frac{(R\sqrt{2}/\lambda)}{2k} \frac{K_0(R\sqrt{2}/\lambda)}{K_1(R\sqrt{2}/\lambda)}. \tag{33}$$

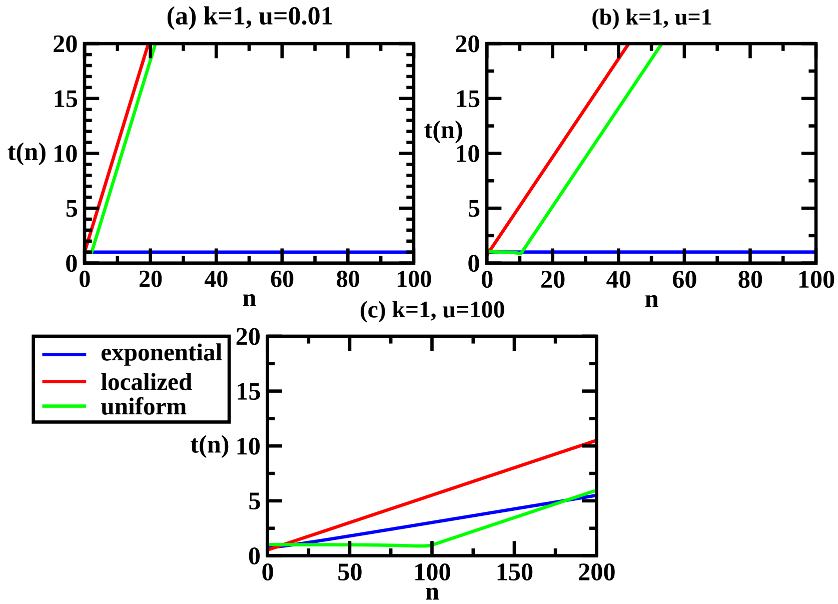


Figure 5. Local accumulation times for the formation of morphogen gradients with different production regions and for variable diffusion and degradation rates. Red curves describe the single-site localized source, green curves describe the uniform production over the finite interval, and blue curves describe the exponential production over the semi-infinite interval. Adapted with permission from Ref. [75].

In the case of $d = 3$, it was shown that [43]

$$C_{s,d=3}(r) = \frac{QR^2 \exp\left[(R-r)\sqrt{3}/\lambda\right]}{rD(1+R\sqrt{3}/\lambda)}, \tag{34}$$

and

$$\tau_{d=3}(r) = \frac{(r-R)\sqrt{3}/\lambda}{2k} + \frac{R\sqrt{3}/\lambda}{2k(1+R\sqrt{3}/\lambda)}. \tag{35}$$

The analysis of the dynamics of the formation of morphogen gradients in two and three dimensions led to some unexpected results [43]. It was found that, in contrast to one-dimensional systems, there are multiple time scales for approaching the stationary concentration profiles near the production region ($r \simeq R$). It was suggested then that the dimensionality is an important factor in the morphogen gradients development in multi-dimensional systems, although the mechanisms of this phenomenon were not clarified [43].

These surprising observations were fully explained only when more general multi-dimensional discrete-state stochastic models were introduced [60]. The d-dimensional system with the production at the origin, as shown in Fig. 6, was investigated. In this system, each lattice cite is characterized by d coordinates, $\vec{\mathbf{n}} = (n_1, n_2, ..., n_d)$. The source of signaling molecules is at the origin, $\vec{\mathbf{n}}_0 = (0, 0, ..., 0)$, with the production rate Q (see Fig. 6). Morphogens can diffuse to the nearest neighboring sites with the rate u, and the degradation rates at each site is equal to k (Fig. 6). To solve the problem, a function $P(n_1, n_2, ..., n_d; t)$, defined as the probability density at the cell $\vec{\mathbf{n}} = (n_1, n_2, ..., n_d)$ at time t, was analyzed at all times using the following master equations [60],

$$\frac{dP(n_1, n_2, ..., n_d; t)}{dt} = u \sum_{nn} P(n_1, n_2, ..., n_d; t) - (2ud + k) P(n_1, n_2, ..., n_d; t), \quad (36)$$

where $\sum_{nn}$ corresponds to summing over all nearest neighbors,

$$\sum_{nn} P(n_1, n_2, ..., n_d; t) = P(n_1 - 1, n_2, ..., n_d; t) + P(n_1 + 1, n_2, ..., n_d; t) + P(n_1, n_2 - 1, ..., n_d; t) + P(n_1, n_2 + 1, ..., n_d; t) + ... \quad (37)$$

At the origin, the dynamics is slightly different,

$$\frac{dP(0, 0, ...; t)}{dt} = Q + u \sum_{nn} P(0, 0, ...; t) - (2du + k) P(0, 0, ...; t). \quad (38)$$

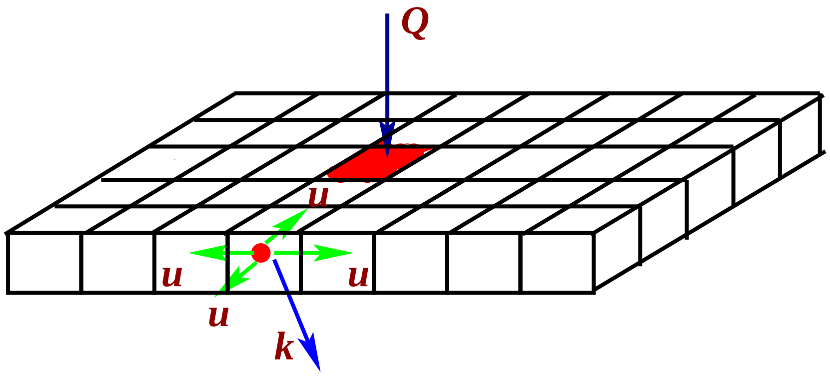


Figure 6. A schematic view of the multi-dimensional system with the formation of morphogen gradients for $d = 2$. The signaling molecules are created at the origin with the rate Q. They diffuse with the rate u in all directions without a bias. At each cell, morphogens can be degraded with the rate k. Adapted with permission from Ref. [60].

At large times, the system achieves a stationary state with exponentially decaying signaling concentration profile,

$$P^{(s)}(n_1, n_2, ..., n_d) = \frac{2Q x^{|n_1| + |n_2| + ... + |n_d|}}{\sqrt{k^2 + 4duk}} = \frac{2Q}{\sqrt{k^2 + 4duk}} \exp\left(\frac{- \mid n_1 \mid - \mid n_2 \mid - ... - \mid n_d \mid}{\lambda}\right), \quad (39)$$

where

$$x = (2du + k - \sqrt{k^2 + 4duk})/(2du), \quad \lambda = -1/\ln x. \quad (40)$$

It was shown also that the dynamics of approaching the stationary state is specified by the LAT [60]

$$t(n_1, n_2, ..., n_d) = \frac{(2du + k)}{(k^2 + 4duk)} + \frac{\mid n_1 \mid + \mid n_2 \mid + ... + \mid n_d \mid}{\sqrt{k^2 + 4duk}}. \tag{41}$$

The equivalent expression for the LAT at the distance r from the origin produces [60],

$$\tau(r) = \frac{(2du + k)}{(k^2 + 4duk)} + (\frac{\sqrt{d}}{\sqrt{k^2 + 4duk}})r. \tag{42}$$

In the fast degradation limit, $k \gg u$, this equation gives

$$\tau(r) \simeq \frac{1}{k} + \frac{r\sqrt{d}}{k}. \tag{43}$$

In the continuum limit, $u \gg k$, it was found that there is no dependence on the dimensionality [60],

$$\tau(r) \simeq \frac{1}{2k} + \frac{r}{2\sqrt{uk}}. \tag{44}$$

This contrasts with the predictions of the radially-symmetric continuum models [43,44]. From Eq. (31) one could obtain for the localized source $R = 0$,

$$\tau_d(r) = \frac{1}{k} + \frac{(r\sqrt{d}/\lambda)}{2D} \frac{K_{d/2}(r\sqrt{d}/\lambda)}{K_{d/2-1}(r\sqrt{d}/\lambda)}. \tag{45}$$

To rationalize these deviations between theoretical predictions, the LAT in two and three dimensions for both approaches have been compared. The results are presented in Figs. 7 and 8. One can see that the continuum limit of the discrete-state models and radially-symmetric continuum models do not agree with each other, although for large distances ($r \gg 1$) the differences are getting smaller [60]. It was noticed also that the radially-symmetric models predict that $\tau(r = 0) = 0$, while for the discrete-state case $\tau(r = 0) = 1/2k \neq 0$. But the relaxation times to the stationary profiles can never be zero because originally in the system there is no morphogens. Thus, the radially-symmetric continuum models cannot properly describe the dynamics of the formation of morphogen gradients for $d > 1$ near the production region. The main reason for this is the assumption of spherically symmetric solutions of the corresponding reaction-diffusion equations at all length scales. Theoretical approach based on the discrete-state stochastic framework does not assume the spherical symmetry and this allows to correctly describe the dynamics at all scales and in all dimensions [60].

The effect of dimensionality on the dynamics of the formation of morphogen gradients have been also investigated using the discrete-state stochastic models [60]. The results are presented in Figs. 9 and 10. It was found that the dynamics is determined by the distance from the source and by the relative values of the degradation and diffusion rates. LAT depend on d for fast degradation rates ($k \gg u$), while there is no dependence in the continuum limit($u \gg k$). The last observation can be simply explained by noting that in the continuum case the diffusion rate is very fast and the rate-limiting step is the production of morphogens, which is clearly independent of the

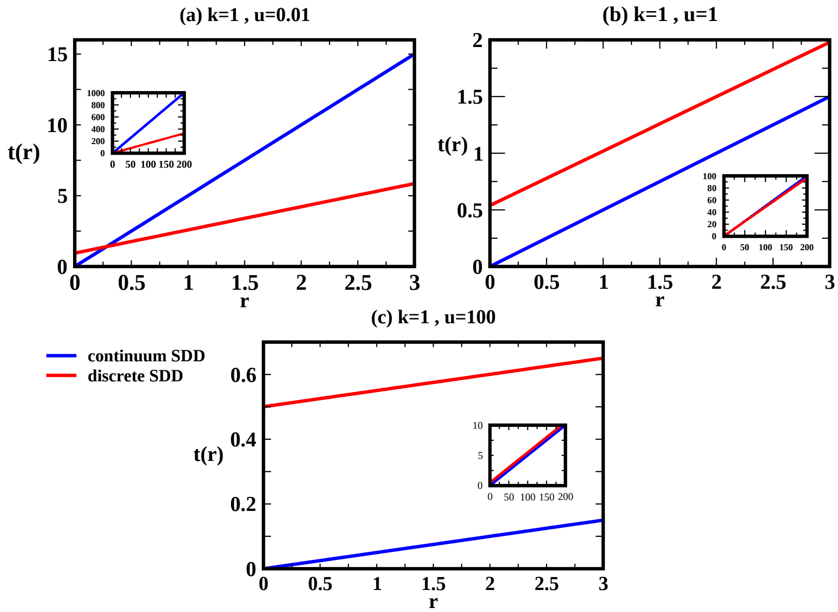


Figure 7. Comparison of the LAT for discrete-state stochastic models and for radially-symmetric continuum models as a function of the distance from the source r in two dimensions. a) Fast degradation limit, $k = 1$, $u = 0.01$; b) comparable diffusion and degradation rates, $k = u = 1$; and c) fast diffusion limit, $k = 1$, $u = 100$. Insets show the same plots for larger length scales. Adapted with permission from Ref. [60].

dimension. Theoretical calculations also show that the LAT for the sites far away from the source ($r \gg 1$) increase with d, while for the sites near the production area ($r \simeq 0$) the trend is reversed: see Figs. 9 and 10. The following arguments were presented to explain these results [60]. There are two effects by which the dimension affects the dynamics of morphogen molecules. Increasing d effectively increases the mobility of the signaling molecules because there are more channels to escape from the given site. At the same time, there are more pathways that connect the source region and any other site on the lattice, and this should increase the relaxation times because there are more long slow trajectories connecting the source and the target cell. The first effect dominates at small distances near the source because there are less pathways to reach the given cell. At the same time, the second effect is more important for large distances.

To investigate in more detail the dynamics of the formation of signaling profiles, higher moments of the relaxation to the stationary states have been calculated [44, 60]. From this point of view, the LAT is the first moment, $\tau \equiv < t >$. Using the discrete-state stochastic method, the variance in the local accumulation times, $\sigma \equiv \sqrt{< t^2 > - < t >^2}$,

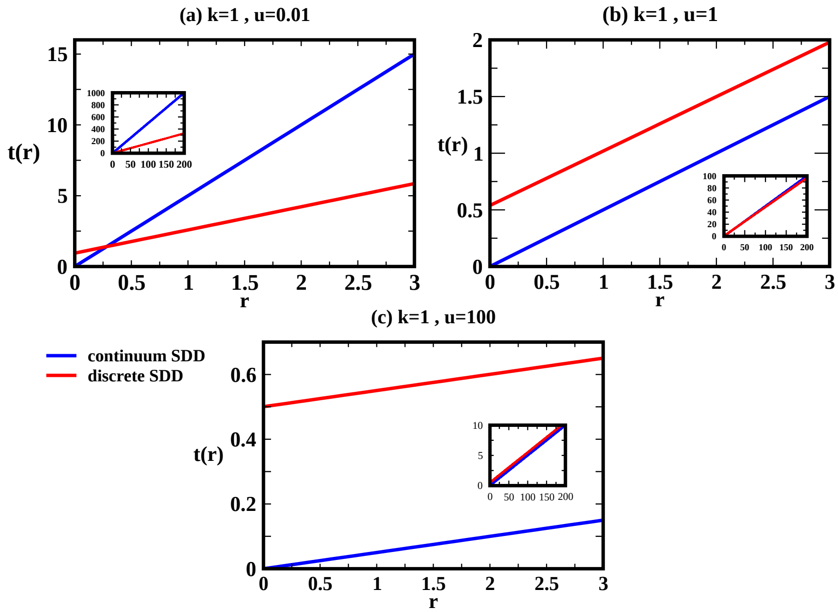


Figure 8. Comparison of the LAT for discrete-state stochastic models and for radially-symmetric continuum models as a function of the distance from the source r in three dimensions. a) Fast degradation limit, $k = 1$, $u = 0.01$; b) comparable diffusion and degradation rates, $k = u = 1$; and c) fast diffusion limit, $k = 1$, $u = 100$. Insets show the same plots for larger length scales. Adapted with permission from Ref. [60].

was estimated as [60]

$$\sigma(r) = \left[\frac{dr^2 - 2}{(k^2 + 4dk)} + \frac{2r\sqrt{d}(2du + k)}{(k^2 + 4duk)^{3/2}} + \frac{5(2du + k)^2}{(k^2 + 4duk)^2} \right]^{1/2}. \tag{46}$$

In the limit of fast degradation rates, $k \gg u$, this expression simplifies into

$$\sigma(r) \simeq \frac{\sqrt{dr^2 + 2r\sqrt{d} + 3}}{k}, \tag{47}$$

while in the limit of fast diffusion rates (continuum limit) the variance is

$$\sigma(r) \simeq \frac{\sqrt{5}}{2k} + \frac{r}{2\sqrt{5uk}}. \tag{48}$$

These results suggest that, similarly to the LAT, the dependence of the variance of the relaxation times to the stationary profile on the dimension disappears in the continuum limit [60].

The first and second moments of the relaxation times have been further employed in analyzing an important question on how biological systems might control the stochastic noise during the processes of the formation of morphogen gradients [60]. It was argued that the variance normalized by the LAT is a convenient measure of noise, and it is

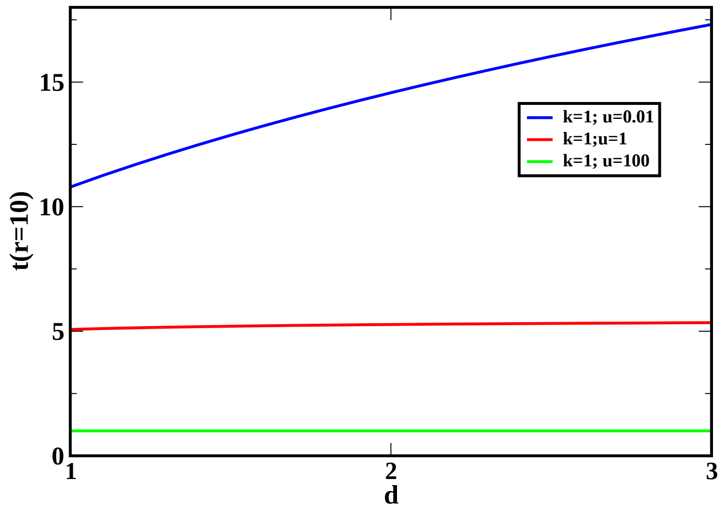


Figure 9. Local accumulation times at $r = 10$ as a function of the spatial dimension. The blue curve corresponds to the fast degradation rates, $k = 1$, $u = 0.01$. The red curve is for comparable degradation and diffusion rates, $k = u = 1$. The green curve describes the fast diffusion limit, $k = 1$, $u = 100$. Adapted with permission from Ref. [60].

presented in Fig. 11. Theoretical calculations show that the noise can be reduced by increasing the degradation rate and the dimensionality of the system.

2.5. Nonlinear Degradation Mechanisms

Several experimental studies suggested that in some systems the development of the signaling profiles might be associated with more complex nonlinear degradation processes [51,54–56]. In these situations, the presence of other morphogens can catalyze or inhibit the process of the removal from the medium, and this should affect the dynamics of the formation of morphogen gradients. In this case, the temporal evolution of the concentration profile can be written as [51]

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2} - kC(x,t)^m, \tag{49}$$

with $m \neq 1$. Using numerical solutions and mathematical bounds initial studies have shown that the dynamics of approaching to the stationary state differs significantly depending on the parameter m [51]. For $m = 0$ and $m = 1$ (linear degradation) LAT are linear functions of the distance from the source, but for $m = 2$, 3 and 4 the scaling changes from linear to quadratic.

The explanations for these surprising observations were presented in the theoretical analysis that proposed to view the degradation process as an effective driving potential [76]. The degradation creates a gradient by removing molecules from the system, and

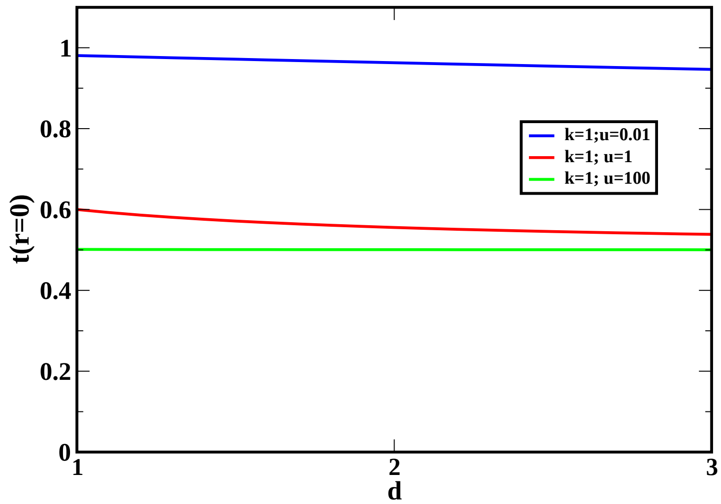


Figure 10. Local accumulation times at $r = 0$ as a function of the spatial dimension. The blue curve corresponds to the fast degradation rates, $k = 1$, $u = 0.01$. The red curve is for comparable degradation and diffusion rates, $k = u = 1$. The green curve describes the fast diffusion limit, $k = 1$, $u = 100$. Adapted with permission from Ref. [60].

this is equivalent to the action of the potential given in Eq. (15) that drives molecules away from the production region. Then the original reaction-diffusion model with the degradation can be approximated as a biased-diffusion model without degradation [76]. This is illustrated in Fig. 12.

It was assumed that the equivalent biased-diffusion model has L ($L \to \infty$) sites [76]. The morphogens starts the motion at $t = 0$ at the origin. The particles can move to the right (left) from the site n with the rate $g_n(r_n)$: see Fig. 12. When the particle reaches the site L it is instantaneously moved back to the origin, $n = 0$. The model is non-equilibrium, so that there is always a flux in the system in the direction away from the source. In the biased-diffusion model the probability to find the molecule at the site n at time t is given by a function $\Pi_n(t)$ [76]. The temporal evolution of these probability is described by master equations [76],

$$\frac{d\Pi_n(t)}{dt} = r_{n+1}\Pi_{n+1}(t) + g_{n-1}\Pi_{n-1}(t) - (r_n + g_n)\Pi_n(t), \tag{50}$$

for $0 < n < L$, while for $n = 0$ and $n = L$, we have

$$\frac{d\Pi_0(t)}{dt} = J + r_1\Pi_1(t) - g_0\Pi_0(t), \tag{51}$$

$$\frac{d\Pi_L(t)}{dt} = g_{L-1}\Pi_{L-1}(t) - r_L\Pi_L(t) - J, \tag{52}$$

where J is the flux from the site L back to the origin $n = 0$. When the system achieves the stationary-state behavior the flux through every site is equal to J.

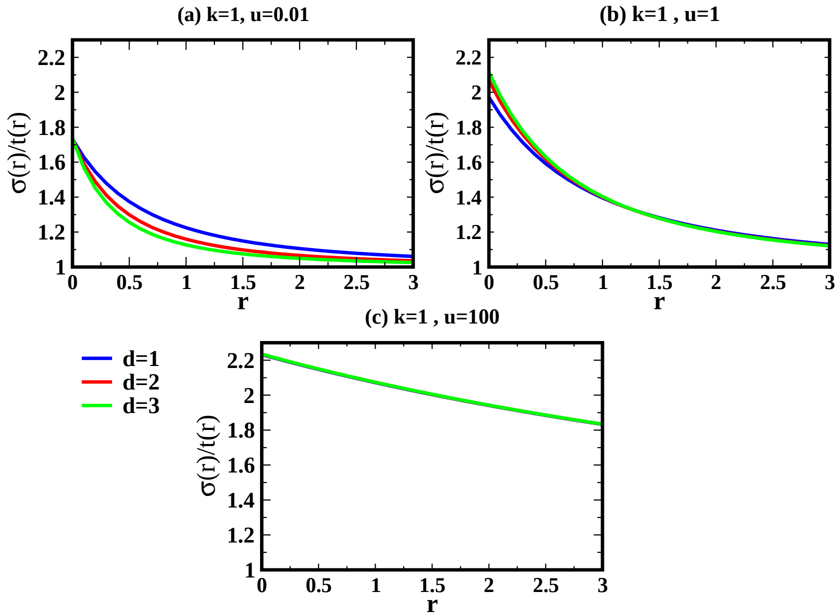


Figure 11. Normalized variance as a function of distance from the source for different dimensions in the discrete-state stochastic models. a) Fast degradation rates, $k = 1$, $u = 0.01$; b) comparable degradation and diffusion rates, $k = u = 1$; and c) fast diffusion rates, $k = 1$, $u = 100$. Adapted with permission from Ref. [60].

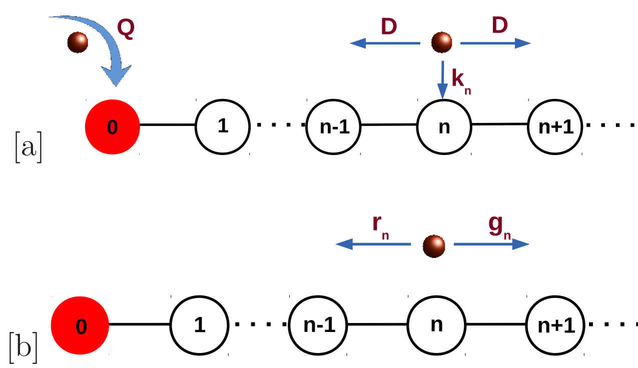


Figure 12. Schematic view of equivalent models for the formation of morphogen gradients. a) Synthesis-diffusion-degradation model; b) Biased-diffusion model. Adapted with permission from Ref. [76].

Comparing the SDD model and the equivalent biased-diffusion model, it should be clear that the mapping between them is not exact [76]. It can be seen by noting that in the biased-diffusion model there is always a conservation of the probability, while in the SDD model the conservation is only achieved at the stationary-state limit. The relations between the parameters of both models can be made quantitative by using the following arguments. The diffusion rates g_n and r_n are related to each other via the effective potential as can be shown using the detailed balance-like arguments [76, 82],

$$\frac{g_n}{r_{n+1}} = \exp\left(\frac{U_n^{eff} - U_{n+1}^{eff}}{k_{\mathrm{B}}T}\right). \tag{53}$$

The physical meaning of this expression is that the stronger the potential, the faster the motion in the positive direction, $g_n > r_{n+1}$. But to obtain the explicit formulas for transition rates a second condition is needed [76],

$$g_n + r_n = 2D + kC^{m-1}. \tag{54}$$

This implies that the residence of each molecule at site n is identical in both models. Together, Eqs. (53) and (54) uniquely define the transition rates in the biased diffusion model via parameters of the SDD model [76].

For linear degradation ($m = 1$) this approach leads to the following transition rates in the equivalent biased-diffusion model [76],

$$g_n = g = \frac{2D + k}{x + 1}, \quad r_n = r = x\frac{2D + k}{x + 1}, \tag{55}$$

with $x = (2D + k - \sqrt{k^2 + 4kD})/2D$. The results for mean first-passage times from both models are given in Fig. 13. We conclude that the approximate mapping works quite well everywhere, but especially for large degradation rates.

Extending this method to nonlinear degradation processes, indicates that for $m \geq 2$ the steady-state profile is given by [76],

$$P_n^{(s)} \simeq \frac{1}{(1 + n/\lambda)^{\frac{2}{m-1}}}, \tag{56}$$

where the parameter λ is defined as

$$\lambda = \frac{1}{m-1}\left[\frac{(2D)^m(m+1)}{kQ^{m-1}}\right]^{\frac{1}{m+1}}. \tag{57}$$

This concentration profile corresponds to the logarithmic potential,

$$\frac{U_n^{eff}}{k_B T} \simeq -\frac{2}{m-1}\ln(1 + n/\lambda). \tag{58}$$

It can be shown that the mean first-passage times for equivalent the biased-diffusion model at large distances from the source are equal to [76]

$$\tau_n \simeq \frac{(m-1)}{(m+1)}\frac{n^2}{2D}. \tag{59}$$

This is an important result since it predicts a quadratic scaling for relaxation times with the nonlinear degradation, as illustrated also in Fig. 14.

Theoretical calculations using the mapping of the SDD model to the equivalent biased-diffusion model clearly show different scaling behavior depending on the mechanisms of degradation. Linear scaling is observed for $m = 0$ or 1, while quadratic

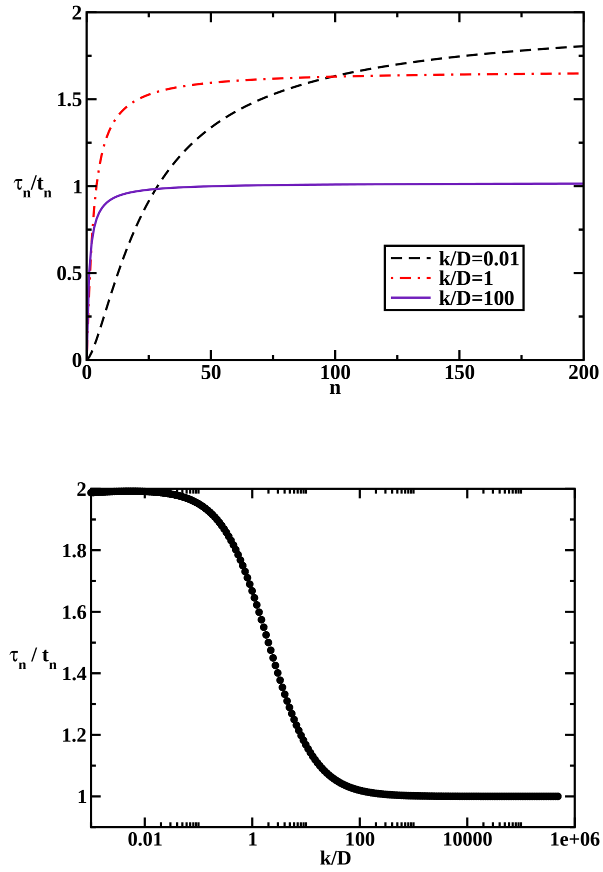
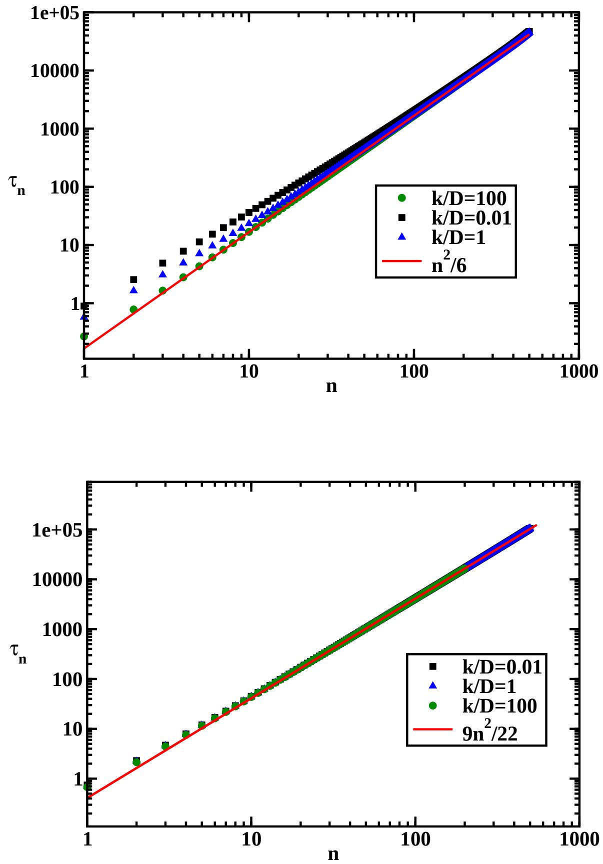


Figure 13. Ratio of calculated mean first passage times for the SDD model and for the equivalent biased-diffusion model. a) The dependence on the distance from the source; b) the dependence on the ratio of degradation rate over the diffusion rate. Distance from the source is set to $n = 10^4$. Adapted with permission from Ref. [76].

scaling is found for nonlinear degradations with $m \geq 2$ [51, 76]. The different dynamic behavior was explained using the concept of the effective potentials due to degradation [57]. Linear degradation corresponds to strong driving potential, as shown in Fig. 15. In this case, there is a unique length scale λ across the whole system. This leads to effectively driven diffusion which has the expected linear scaling. The situation is different for the nonlinear degradation processes. The stationary state in this case can be described by a power-law concentration profiles, which do not possess unique length scales. As a result, the effective potential (logarithmic) is weak enough so that it cannot destroy the quadratic scaling of the unbiased diffusion. It might only affect the amplitude of the random-walk fluctuations for each signaling molecule.



[b

Figure 14. Theoretically calculated mean first-passage times as a function of the distance from the source for different degrees of nonlinearity for the biased-diffusion model. a)$m = 2$; b) $m = 10$. Adapted with permission from Ref. [76].

2.6. Alternative Mechanisms: Direct Delivery of Morphogens

Recent experimental advances in studying the development processes in various systems revealed that there is a significant number of experimental observations that cannot be explained by reaction-diffusion mechanisms [11, 47, 64, 65, 69]. In embryo systems with complex internal structures simple free diffusion might not be always very efficient in establishing the morphogen gradient [11, 64]. These observations stimulated new ideas on how the genetic information can be transferred in such systems. An alternative direct delivery mechanism has been proposed [11, 41, 63, 66]. It was suggested the signaling molecules can be transported to target cells utilizing cellular tubes, which are called cytonemes [11, 41, 63, 66, 69]. Cytonemes are dynamic cellular extensions that cells can extend and retract very quickly with the help of actin filaments. Their length is varying from 1 to 100 μm with the diameter of less than 100 nm. Cytonemes have been recently observed in several biological systems, but their cellular functions were

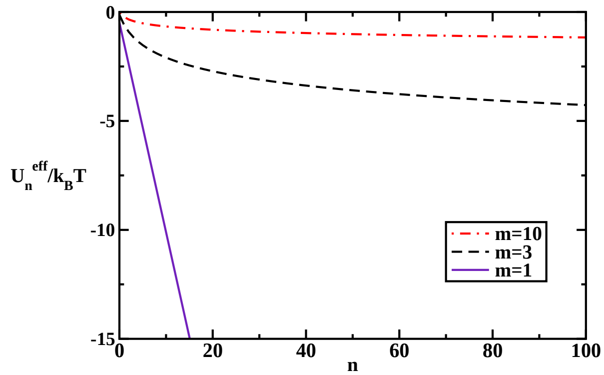


Figure 15. Effective potentials due to degradation. Linear degradation corresponds to $m = 1$, while $m = 3$ and $m = 10$ describe non-linear degradation processes. Adapted with permission from Ref. [76].

unclear [63–65, 68–71, 74]. It was proposed that morphogens can be transported by myosin motor proteins along the actin filaments inside the cytonemes directly from the source cells to the target cells, as shown schematically in Fig. 16 [11, 68, 69]. The direct delivery mechanism thus avoids the problems where geometrically complex environment prevents the free diffusion to form the signaling profiles.

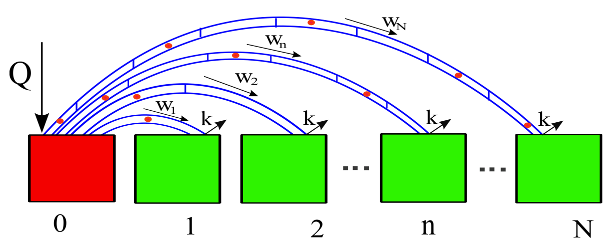


Figure 16. A simplified view of the direct delivery mechanism of signaling molecules via utilization of cytonemes. The red cell is the source. Green cells labeled as $n = 1, ..., N$ are target cells. Cytonemes are shown as tubular extensions from the source cell to the target cells. Morphogens are small red circles inside the cytonemes. Adapted with permission from Ref. [77].

2.7. Transport through Cytonemes

Recently, a first quantitative physical-chemical method to describe the direct delivery via cytonemes has been introduced [77]. It is based on discrete-state stochastic analysis of the model presented in Fig. 16. The model assumes that there are $N + 1$ embryo cells in the system (shown as squares in Fig. 16). One of them (red square, $n = 0$) is a special source cell where the signaling molecules are produced with a rate Q. The source cell also generates N cytonemes that extend and attach to each of the target cell (green squares in Fig. 16). It is assumed that the cytonemes are already established at the beginning of the process and they are stable until the morphogen gradient is fully established. Signaling molecules (shown as small red circles in Fig. 16) are transported to the n-th target cell from the source cell with a rate w_n ($n = 1, 2, ..., N$). When they reach their target cells, morphogens can be degraded with a rate k. This is the minimal model that takes into account the most relevant processes such as the direct delivery via cytonemes and the degradation of morphogens.

This model can be solved by analyzing the single-molecule probability density function $P_n(t)$ of finding the morphogen at the site n at time t. The temporal evolution of this probability function follows the set of master equations [77]

$$\frac{dP_0(t)}{dt} = Q - \sum_{n=1}^{N} w_n P_0(t), \tag{60}$$

for $n = 0$, and

$$\frac{dP_n(t)}{dt} = w_n P_0(t) - kP_n(t) \tag{61}$$

for $n > 0$. Assuming that initially there were no morphogens in the system, $P_n(t = 0) = 0$ for all n, these master equations can be solved exactly at all times, which leads to

$$P_0(t) = \frac{Q}{\eta}\left[1 - e^{-\eta t}\right]; \tag{62}$$

$$P_n(t) = \left[\frac{Qw_n}{\eta(\eta - k)}\right] e^{-\eta t} - \left[\frac{Qw_n}{k(\eta - k)}\right] e^{-kt} + \frac{Qw_n}{\eta k}, \tag{63}$$

where $\eta = \sum_{n=1}^{N} w_n$ is defined as a total productions rate from the source cell to all target cells. These results imply that the concentration of signaling molecules at each cell is an exponentially decaying function of the time. It can be viewed as a result of balancing between two opposing processes: the direct delivery with the rate η and the removal with the rate k. In the stationary-state limit, ($t \to \infty$), the density profiles reduce to,

$$P_n^{(s)} = \frac{Q}{k\eta} w_n, \quad P_0^{(s)} = \frac{Q}{\eta}. \tag{64}$$

The dynamics of approaching the stationary state can be understood from analyzing a local relaxation function, defined as $R_n(t) \equiv \frac{P_n(t) - P_n^{(s)}}{P_n(0) - P_n^{(s)}}$ [34]. Simple calculations yield the following expressions for the local accumulation times [77],

$$< \tau_n >= \frac{1}{k} + \frac{1}{\eta}, \quad < \tau_0 >= \frac{1}{\eta}. \tag{65}$$

The model predicts that there is no dependence on the target cell position, n, in contrast to reaction-diffusion mechanisms of the formation of morphogen gradients. The relaxation dynamics to the stationary concentration profiles is identical for all target cells. The main reason for this is that the processes at all target cells are independent from each other, and the stationary state at each of them cannot be established until the steady-state behavior is observed in the source cell [77]. This can only happen simultaneously in all cells in the system (see Fig. 16).

Local relaxation functions have been also applied to obtain the second moment of LAT, which allowed to evaluate the robustness of the direct-delivery mechanism [77]. It was shown that

$$< \tau_n^2 >= \frac{2(\eta^2 + k\eta + k^2)}{k^2\eta^2}, \quad < \tau_0^2 >= \frac{2}{\eta^2}, \tag{66}$$

which are again independent of the position of the target cell, n. The normalized variance was then computed to be [77],

$$\sigma_n = \left[\frac{\eta^2 + k^2}{\eta^2 + 2k\eta + k^2}\right]^{1/2}, \quad \sigma_0 = 1. \tag{67}$$

The normalized variances for the direct-delivery mechanisms are compared with the corresponding predictions from the reaction-diffusion processes in Fig. 17. One can see that σ_n is always less for the direct delivery transport. This means that moving signaling molecules through cytonemes is a more robust mechanism of the formation of morphogen gradients because it is affected less by the stochastic noise [77]. In the reaction-diffusion mechanism signaling molecules can fluctuate spatially between different cells due to diffusion, but this option is not available for the direct-delivery mechanism. So the advantage of using the transport via cytonemes in creating signaling profiles is not only in overcoming the geometric constraints but also in reducing the influence of the stochastic noise [77].

To understand better how the direct delivery process works, a more microscopic description of the transportation rates w_n was utilized for calculating the dynamic properties of the system [77]. In the first approach, it was suggested to use the fact that motor proteins drive the morphogens along the cytonemes. It was assumed that the rates are related to the free energy difference of moving the signaling molecule from the source cell to the target cell [77],

$$w_n = \exp\left[-\frac{\Delta G(n)}{k_B T}\right], \tag{68}$$

where $\Delta G(n)$ is the energy required to displace the morphogen to the target cell n. One can assume that the length of the cytoneme to the target cell n, L_n is proportional to n, i.e., $L_n = An$, and the motor proteins spend energy ε (in units of $k_B T$) by moving every signaling molecule a distance l. Then the free energy difference can be written as

$$\Delta G(n) = \frac{L_n \varepsilon k_B T}{l} = \frac{An\varepsilon k_B T}{l} = \frac{nk_B T}{a}, \tag{69}$$

where $a = l/A\varepsilon$. The explicit expression for the transportation rate is given by $w_n = \exp\left[-\frac{n}{a}\right]$. This finally leads to the following expression for the stationary

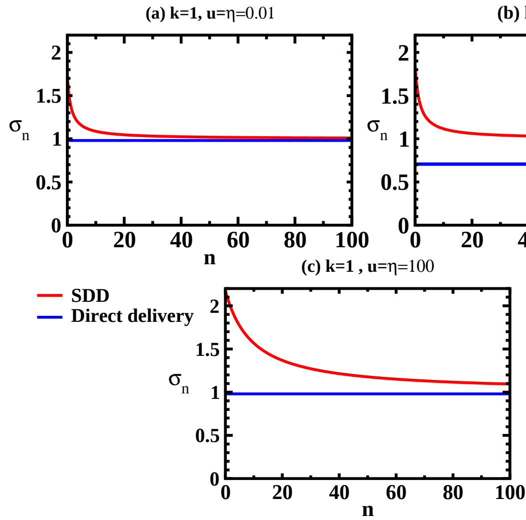


Figure 17. Comparison of normalized variances as a function of the distance from the source for reaction-diffusion and direct delivery mechanisms. Red lines correspond to the SDD model with a diffusion rate u. Blue lines describe the direct delivery via cytonemes with the total transportation rate η. Adapted with permission from Ref. [77].

concentration profile [77],

$$P_n^{(s)} = \frac{Q}{k\eta} \exp\left[-\frac{n}{a}\right], \tag{70}$$

where the total transportation rate η is equal to

$$\eta = \sum_{n=1}^{N} \exp\left[-\frac{n}{a}\right] = \frac{\exp\left[-\frac{1}{a}\right] - \exp\left[-\frac{(1+N)}{a}\right]}{1 - \exp\left[-\frac{1}{a}\right]}. \tag{71}$$

This model predicts the exponential decaying morphogen gradient [see Eq. (70)], which is similar to predictions from the reaction-diffusion models [7–10]. However, the difference is that the decay length in the direct delivery mechanism, specified by the parameter a, is larger for more efficient motor proteins that spend less energy in driving the morphogens along the cytonemes. In the reaction-diffusion mechanism the decay length is controlled by the ratio of diffusion and degradation rates [34, 57]. Thus the energy dissipation in the transportation of signaling molecules through cytonemes is important for direct delivery mechanism [77].

Because cytonemes are narrow cylindrical tubes, the transport of signaling molecules can be viewed as effectively one-dimensional, and this suggested intermolecular interactions, e.g., due to exclusion, might affect the dynamics [77]. This possibility was investigated using the concept of totally asymmetric exclusion processes (TASEP) [77]. TASEPs are nonequilibrium multi-particle models that were successfully utilized for uncovering the mechanisms of many complex biological processes [73]. It was proposed that each cytoneme can be viewed as 1D lattice on which morphogens move in the direction of the target cell. The problem of describing the dynamics of the formation of morphogen gradients in such system is identical to a set of open-boundary TASEP segments coupled at the source cell. Stationary-state fluxes for TASEP on finite lattice segments with an entrance rate α and an exit rate β are well known [72],

$$J(\alpha, \beta; n) = \frac{S_{n-1}(1/\beta) - S_{n-1}(1/\alpha)}{S_n(1/\beta) - S_n(1/\alpha)}, \tag{72}$$

where

$$S_n(y) = \sum_{i=0}^{n-1} \frac{(n-i)(n+i-1)!}{n!i!} y^{n-i+1}. \tag{73}$$

For the model presented in Fig. 17, the entrance and exit rates on each cytoneme are given by [77],

$$\alpha = Q/N, \quad \beta = k. \tag{74}$$

The transition rate from the source cell to the target cell n can be written as

$$w_n = J(Q/N, k; n). \tag{75}$$

The stationary-state profile in this system of interacting morphogens is equal to

$$P_n^{(s)} = \frac{Q}{k\eta} \frac{S_{n-1}(1/k) - S_{n-1}(N/Q)}{S_n(1/k) - S_n(N/Q)}. \tag{76}$$

Fig. 18 illustrates the morphogen gradients for this system of interacting signaling molecules. The possibility of interactions between the morphogen molecules has a dramatic effect on the stationary profiles. While at the distances not far away from the source the effect is minimal, for larger distance the density profile saturates. But this leveling is not useful for the morphogen gradients because the information can be transferred efficiently only from strongly decaying profiles. It was suggested that these intermolecular interactions might present a problem for the direct delivery mechanism on very large distances, but experimental tests of these predictions are needed because many other factors might change the outcome [77].

3. Concluding Remarks and Future Directions

To conclude, we presented a review of recent developments in theoretical understanding the mechanisms that lead to the formation of biological signaling profiles. The dynamics of formation of morphogen gradients was analyzed first using the reaction-diffusion framework. This is assumed to be the main mechanisms for creating the concentration profiles of signaling molecules that can efficiently transfer the information in embryo systems. We discussed the critical role of the degradation processes, and it was argued that its action is similar to the driving potential that accelerates the dynamics of formation of morphogen gradients. Several other important aspects of the

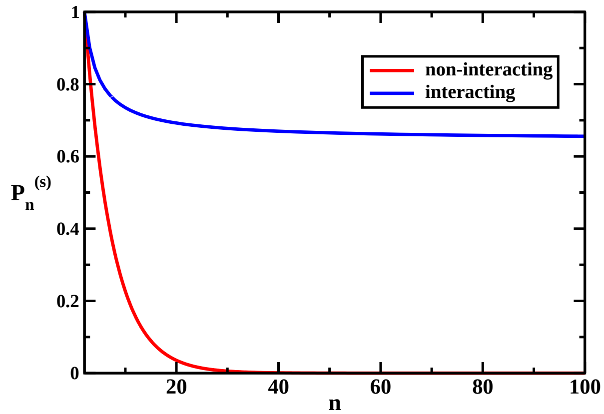


Figure 18. Stationary-state concentration profiles for interacting and non-interacting signaling molecules during the transportation along the cytonemes. Adapted with permission from Ref. [77].

development of signaling profiles, including the effect of the size of the production region, dimensionality, nonlinearity in the degradation and discreteness of these processes, have been thoroughly analyzed. We also discussed the alternative direct delivery mechanisms in the establishment of morphogen gradients. The presented theoretical methods are applicable to a broad range of biological development phenomena, as well as for cell signaling and tissue and organ formation processes.

Although many features of the development of signaling profiles are better understood now, there are many puzzling questions and observations in the field. Let us briefly mention several of them. The production of the morphogens is a time-dependent process with variable rates. But existing theoretical methods mostly assume that these rates are constant. It is not clear how to take into account the temporal effect in the source and what effect it might have on dynamics. Another challenging problem is if the morphogen gradient needs to reach the stationary state or not in order to properly transfer the information. There are controversial views about the possibility of the pre-steady state decoding as the more efficient mechanism of information transfer [23, 58]. It is important to understand this because it might affect the dynamics and the robustness of the system. Another critical question is related to the fact that embryo cells during the formation of morphogen gradients are not frozen as implicitly assumed in current theoretical models. They are dynamic systems that can grow, shrink, divide and change the shape. New theoretical ideas are needed in order to couple the chemical and biophysical processes of the formation of morphogen gradients with mechanical stability and transformations in embryo cells. Finally, it is still unclear how exactly the embryo cells read the information from the signaling profiles. Several ideas were expressed but none of them is fully supported by existing experimental data [47]. It is critically important to combine multiple theoretical, computational and experimental methods to advance our knowledge on mechanisms of these fundamental biological processes.

Acknowledgments

The work was supported by grants from the Welch Foundation (C-1559), from the NSF (Grant CHE-1360979) and by the Center for Theoretical Biological Physics sponsored by the NSF (Grant PHY-1427654).

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