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LETTER

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Formation of a Morphogen Gradient: Acceleration by Degradation

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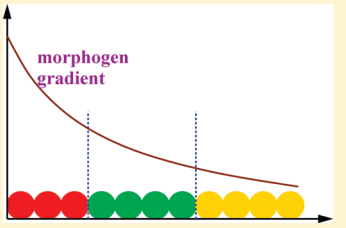
ABSTRACT: Concentration profiles of signaling molecules, known as morphogen gradients, determine polarity and spatial patterning in the development of all multicellular organisms. A widely used approach to explain the establishment of morphogen concentration gradients assumes that signaling molecules are produced locally, then spread via a free diffusion along the line of developing cells and degraded uniformly. However, recent experiments have produced controversial observations concerning the feasibility of this theoretical description. Some experimentally measured dispersions for morphogens cannot support fast formation of stationary concentration profiles. In addition, the latest theoretical analyses of times to establish the morphogen gradient yield a surprising linear scaling as a function of length from the source that is not expected for the unbiased diffusion process. We propose here a theoretical approach that provides a possible physical−chemical mechanism to explain these observations. It is argued that relaxation times to establish morphogen gradients are mostly determined by first arrival times, and the degradation plays a critical role in this mechanism by effectively accelerating diffusion of signaling molecules via removal of slow moving particles. This coupling between diffusion and degradation is analogous to the action of the effective field that drives particles away from the local source.



SECTION: Biophysical Chemistry

It is known that several classes of signaling molecules stimulate complex concentration-dependent responses that are critical for growth, development, and tissue formation in multicellular organisms.[1−4,11] In recent years there have been many quantitative studies that revealed important features and details of morphogen gradients in different biological systems.[5−10] However, fundamental mechanisms of morphogen gradient formation are still not well understood.

The current view of the development of morphogen gradients suggests that this process is a result of a combination of physical and chemical processes. The simplest and the most popular proposed mechanism, known as a synthesis−diffusion−degradation (SDD) model,[5] assumes that locally produced signaling molecules diffuse freely along the tissue while being uniformly degraded.[7,8,11] This model has been used successfully to describe temporal evolution of signaling molecule profiles in different biological systems.[7,8,10,12] However, the application of this mechanism to the formation of bicoid morphogen gradient led to controversial observations.[5,6] Measured mobility of bicoid molecules was too low to explain fast establishment of the stationary-state profile by a simple unbiased diffusion. Several ideas how to resolve this paradox, such as biased diffusion due to active processes[6] and the effect of the advective transport,[13] have been proposed. However, experimentally all these suggestions have not yet been supported. Furthermore, a recent theoretical study of the formation of morphogen gradients has provided a systematic approach to explicitly evaluate time to reach steady-state concentration profiles.[14] The most surprising observation of this theoretical analysis is a linear scaling as a function of the distance from the source for the time to establish a morphogen gradient in the SDD model. It led to a conclusion that stationary concentration profiles could be formed faster than was thought previously, but the mechanism of such acceleration remains unclear. In the system with the unbiased diffusion, a quadratic scaling with the distance and slow reaching of steady-state conditions are expected. In this work, we propose a microscopic model that allows one to explain this paradoxical behavior. Our theoretical picture provides a physical−chemical mechanism for the fast formation of morphogen gradients and linear scalings in the relaxation times.

To analyze the formation of the morphogen gradient, we utilize a discrete-state version of the SDD model, as shown in Figure 1. The signaling molecule can be found in one of the discrete sites n ($n \geq 0$) that might be associated with an underlying line of cells. It is analogous to a compartmental model developed recently for the bicoid gradient.[15] In addition, a single-molecule view of the process is adopted here, i.e., the concentration of molecules is equivalent to the probability of finding a single particle at a given site. We assume that particles are produced at the origin, $n = 0$, with a rate Q, then they spread to the right ($n \geq 0$) via a free diffusion along the lattice of discrete sites with a diffusion rate u (see Figure 1). At any position, the particle might also be degraded with a rate k. The continuum description of the system is obtained when the diffusion rate is much faster that the degradation processes, i.e., $u \gg k$. In the discrete case, the probability $P_n(t)$ of finding the particle at the position n at time t is governed by a set of master equations:

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

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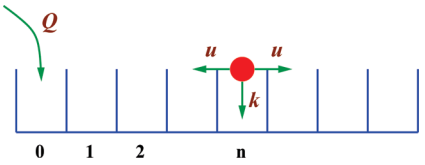

Figure 1. Schematic picture for the discrete-state model of the morphogen gradient formation. Particles are created with the rate Q at the origin. They can also diffuse along the lattice with the rate u, or they might be degraded with the rate k.

for $n > 0$, and

$$\frac{dP_0(t)}{dt} = Q + uP_1(t) - (u+k)P_0(t) \tag{2}$$

At steady-state regime we have $dP_n(t)/dt = 0$, and these differential equations simplify into a system of algebraic equations that can be analytically solved. It can be shown that at $t \to \infty$ the stationary profile is an exponentially decaying function of the distance from the source, and it is given by

$$P_n^{(s)} = \frac{2Qx^n}{k + \sqrt{k^2 + 4uk}} \tag{3}$$

with $x = (2u + k - (k^2 + 4uk)^{1/2})/(2u)$. The corresponding decay length of the concentration profile is equal to $\lambda = -1/\ln x$. In the case when diffusion is faster than the degradation, $u \gg k$, we obtain $\lambda \simeq (u/k)^{1/2}$, which is a well-known result from the continuum SDD model.[14] For fast degradation rates, $k \gg u$, the decay length, as expected, is much smaller, $\lambda \simeq 1/\ln(k/u)$. In this case, morphogens are removed so fast that they cannot diffuse far away from the source.

Applying the theoretical analysis of Berezhkovskii and coworkers[14] we can calculate the time to reach the stationary-state profile at any site by evaluating the Laplace transform of the local relaxation function $R_n(t) = 1 - (P_n(t))/(P_n^{(s)})$. The relaxation time is given by $t_n \simeq \tilde{R}(s = 0)$, and it yields for the discrete-state SDD model

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

One can see that the linear scaling is found again. For slow degradation rates, $k \ll u$, it can be shown that

$$t_n \simeq \frac{1}{2k}\left[1 + \frac{n+1}{\sqrt{u/k}}\right] \tag{5}$$

which is exactly the relation obtained in the continuum SDD model.[14] In the regime of slow diffusion, $u \ll k$, the relaxation time depends only on the degradation rate, $t_n \simeq (n+1)/k$. In contrast to naive expectations, for all ranges of parameters, increasing the degradation rate accelerates reaching the stationary-state profile. One might come to the important conclusion here that fast formation of morphogen gradients and linear scalings are not artifacts of the continuum approximation of underlying biochemical processes.

To understand the mechanisms of fast relaxation, we should recall that initially at $t = 0$ the particle is at the position $n = 0$, and to achieve a steady-state profile at the site n it must first reach this site. Thus we hypothesize that a total time to establish a morphogen gradient at the site n is a combination of the mean first-passage time (MFPT) to arrive to this site, which must strongly depend on n, and a local rearrangement time, which most probably is a weak function of the position. To test this idea, we explicitly calculate MFPTs, τ_n, for the discrete-state SDD model, and we compare them with relaxation times t_n obtained via relaxation functions analysis.[14] It is important to note that in our analysis, conditional MFPTs, which describe the time to reach the site given that the particle survive, are needed. It can be accomplished by analyzing a function $f_n(t)$ defined as a first-passage probability to reach for the first time the site n at time t if at $t = 0$ the particle was at the origin. Temporal evolution of this function follows a system of backward-master equations:[16]

$$\frac{df_n(t)}{dt} = u[f_{n+1}(t) + f_{n-1}(t)] - (2u + k)f_n(t) \tag{6}$$

for $n > 0$, and

$$\frac{df_0(t)}{dt} = uf_1(t) - (u+k)f_0(t) \tag{7}$$

Again using Laplace transformations, $\tilde{f}_n(s) = \int_0^\infty f_n(t)e^{-st}\,dt$, it can be shown that

$$\tilde{f}_n(s) = \frac{2\sqrt{a^2 - 4u^2}\,y^n}{(a - 2u + \sqrt{a^2 - 4u^2})y^{2n} - (a - 2u - \sqrt{a^2 - 4u^2})} \tag{8}$$

where $a = s + 2u + k$, and $y = [a + (a^2 - 4u^2)^{1/2}]/2u$. The conditional mean first-passage time to reach the site n can be found from the following expression:

$$\tau_n = -\frac{\dfrac{d\tilde{f}_n(s)}{ds}_{|s=0}}{\tilde{f}_n(s)_{|s=0}} \tag{9}$$

The explicit formula for MFPT is rather bulky:

$$\tau_n = \frac{1}{\sqrt{k^2 + 4uk}}\left[-\frac{2u + k}{\sqrt{k^2 + 4uk}}\right.$$
$$+\frac{(2u + k + \sqrt{k^2 + 4uk})z^n + (2u + k - \sqrt{k^2 + 4uk})z^{-n}}{(k + \sqrt{k^2 + 4uk})z^n - (k - \sqrt{k^2 + 4uk})z^{-n}}$$
$$\left.+\frac{(k + \sqrt{k^2 + 4uk})nz^n + (k - \sqrt{k^2 + 4uk})nz^{-n}}{(k + \sqrt{k^2 + 4uk})z^n - (k - \sqrt{k^2 + 4uk})z^{-n}}\right] \tag{10}$$

where $z = [2u + k + (k^2 + 4uk)^{1/2}]/2u$. However, it simplifies in the limiting cases: for large degradation rates, $k \gg u$, it gives $\tau_n \simeq (n+1)/k$, while for the fast diffusion along the cells, $u \gg k$, the resulting MFPT is $\tau_n \simeq n/2(ku)^{1/2}$, and this is the result that one obtains from the continuum approach. It is important to note that linear scaling with the distance is *also* observed for first arrival times at all conditions. In addition, in both limiting cases far away from the origin, $n \gg 1$, the expressions for MFPT become equal to the relaxation times as presented above, in agreement with our original idea.

The contributions of MFPT in the overall times to form the stationary-state profile for different diffusion and degradation rates are illustrated in Figure 2. One can see that, in the process of developing the morphogen gradient at the given site, reaching this site for the first time becomes a rate-limiting step, and this effect is stronger the further the site is from the origin. It supports

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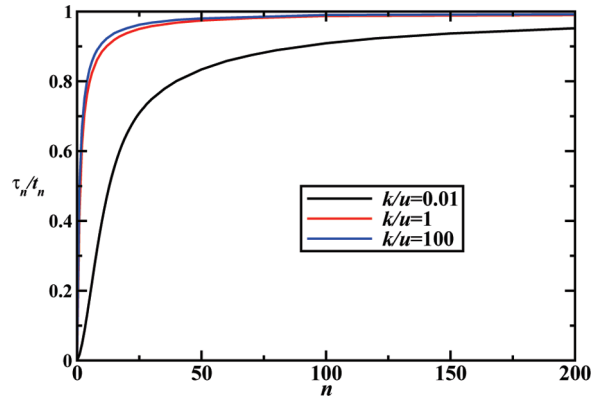

Figure 2. Ratio of the relaxation time to establish the morphogen gradient and the MFPT as a function of the distance from the source for different diffusion and degradation rates.

our hypothesis on the importance of MFPT in the formation of the morphogen gradient. First-arrival times provide a better description of the relaxation times with increasing degradation rates. As shown in Figure 2, MFPT accounts for more that 90% of the relaxation time for $n > 100$ in the case of weak degradation rates ($k/u = 0.01$). For larger degradation rates ($k/u \geq 1$) the deviation between relaxation times and MFPT is less than 10% for much shorter distances from the source ($n > 15$). Thus the time to establish the concentration profile of signaling molecules could be well approximated by the first arrival time. MFPT accounts for almost all relaxation times when the particle is further away from the source, as indicated in Figure 2. It also supports our idea that local rearrangement times depend much more weakly on the distance n than the MFPT.

Comparison of MFPT and relaxation times suggest that mechanisms of morphogen gradient establishment could be understood by analyzing how signaling molecules come first to different regions of the system. Thus the fast formation of steady-state concentration profiles can be attributed to quick arrival of molecules to specific sites. However, it raises a question of how particles move so fast following the linear scaling with the distance in the system without external bias where quadratic scaling is expected. To answer this question, it is important to consider in detail all relevant biochemical and biophysical processes, namely, diffusion and degradation.

In the SDD model particles are not only freely diffusing, but they also have a nonzero probability to be removed from the system by degradation. The residence time of the molecule at each site is a stochastic quantity, and it is decreased by the degradation process. Particles that stay longer at each site will not survive to support the stationary concentration profile. Only molecules with short residence times at each site will remain in the system at large times. This effectively leads to increasing the spreading velocity of molecules, since slower particles will be degraded. The process can also be viewed in the following way: particles in the system with the degradation are the subject of the effective potential $U_{\text{eff}}(n)$ that biases their motion. This can be seen from the fact that the steady-state concentration profile is not uniform, but is rather an exponentially decaying function. This effective potential could be reasonably estimated from the stationary profile, $U_{\text{eff}}(n) \simeq k_B T \ln P_n^{(s)}$. Utilizing eq 3 it can be shown that the effective potential is given by

$$U_{\text{eff}}(n) \sim n \ln x \qquad (11)$$

i.e., there is a constant force ($F \sim \partial U_{\text{eff}}/\partial n$) pushing each particle away from the source. However, the motion of particle in such a strong potential is known as a driven diffusion, which produces linear scaling in the distance as the function of the time. One concludes then that particles in the SDD model cannot be analyzed by the unbiased diffusion approach. The correct theoretical picture of the process is a diffusion in the effective potential created by the degradation. This explains the fast establishment of the morphogen gradient and linear scaling of relaxation times. A surprising result of this theoretical analysis is that the degradation *accelerates* the formation of the stationary profiles by keeping fast surviving molecules and removing slow particles. The effect is similar to the creation of the effective strong potential that drives particles away from the source.

It is also interesting to discuss the biological implications of the acceleration of the gradient formation via this mechanism. While increasing the rate of the signal degradation allows one to establish the stationary concentration profiles faster, less and less particles reach a given location in the cell tissue, and this moves the whole system in the regime with a small number of particles that is characterized by large fluctuations. Thus it might not be beneficiary for the cellular system to increase the degradation rate too much because it also increases noise, which might have negative consequences for the following development processes.

In conclusion, we have proposed a theoretical approach that allows one to explain the fast formation of morphogen gradients and linear scalings with the distance in relaxation times to stationary profiles. Our physical−chemical model argues that in the system with the source production of signaling molecules and the uniform degradation of molecules along the total time to establish the morphogen gradient is a sum of the first-arrival and the local rearrangement times. The steady-state concentration profile of signaling molecules cannot form before particles arrive to the specific sites. It is shown then that the first-passage times control the formation of the morphogen gradient when molecules are far away from the source. The fast molecular motion in such systems is supported by the degradation of particles that remove slow-moving molecules. The degradation effectively creates the effective potential that biases diffusing particles away from the source, and it leads to linear scaling. It is reasonable to suggest that nature could control complex biochemical and biophysical processes of the development by modifying degradation rates and mechanisms. Our theoretical approach could be easily extended to more complex and more realistic models of the morphogen development that include nonlocalized production of signaling molecules, localized degradation region, finite length of the patterned interval, and cooperative mechanisms of degradation. However, it is expected that the presented physical−chemical mechanism will still be valuable for understanding complex biochemical and biophysical processes during the formation of morphogen gradients. It will be important to test the proposed theoretical ideas in experimental studies.

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