# Learn 4D Cell Simulation in 10 pages

**ver. Sep-30-2016**

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# Contents

# Introduction

This is an accompanying eBooklet with "Learn Pathway Simulation in 10 pages". In pathway simulation, interactions between proteins are commonly modeled as a set of biochemical reactions, and time-evolved changes in the protein concentrations are numerically integrated by a computer. Collaborative papers between experimental and pathway simulation are increasing.

In pathway simulation, however, an important aspect in real cells is missing, which is the spatial extent of a cell. A cell is small, but there still exists spatial inhomogeneity within it. For example, a mRNA is reported to be localized at a subcellular compartment during an early embryogenesis. In mature cells, localization of actin regulating proteins in migrating cells is well documented. One prominent example of protein localization is seen in a neuron, where different proteins are localized in different subcellular regions of dendrites, soma, and an axon. These subcellular localization is observed in yeast, drosophila, and in mammals strongly suggesting its functional importance in a cell[1]. In addition, a chemical reaction does not proceed homogeneously within a cell. Different reactions proceed at different subcellular compartments. Distinct reactions proceed in the cytoplasm and in the nucleus, which are easily expected because these are separated by lipid bilayers. Within the cytoplasm, however, different reactions can proceed at different sub-regions in the cytoplasm. These strongly suggest again that intracellular space plays pivotal roles for cellular functioning.

The final goal of simulation in molecular cell biology is the whole cell simulation. This leads to a conclusion as an inevitable consequence, that is, the simulation should include space in a model realizing inhomogeneous distribution of proteins, spatially localized reactions, and interactions between subcellular regions. Cell simulation is the one realizing these. In this eBooklet we discriminate simulation with and without spatial dimensions by calling "cell simulation" and "pathway simulation", respectively[2]. More than 90% of simulations in literatures seem to be pathway simulation. There are several reasons for this: 1) it is not clear what we can obtain more than pathway simulation; 2) no software for cell simulation is found; 3) the analysis of cell simulation is much more difficult than pathway simulation. Point 1) is misunderstanding. We can reach novel findings by cell simulation that is never reached by pathway simulation. An example is shown in Chapter 4. Point 2) is easily solved by using our A-Cell software for cell simulation. Point 3) is true.

By 4D cell simulation, we can reach to unexpected results that are essential for understanding cellular functions and mechanisms. In this eBooklet, we describe bases for 4D cell simulation, which is not difficult. We hope that readers understand the bases for their 4D cell simulation.

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1 e.g. Postma, M., et al., Mol.Biol.Cell, 2003; Pertz, O., et al., Nature, 2006; Thery, M., et al., PNAS, 2006; Shahbabian, et al., Cell.Mol.Life Sci., 2012.

2 We use "4D cell simulation" instead of "cell simulation" to discriminate those from pathway simulation more clearly. 4D = temporal dimension (1D) + spatial dimension (3D).

# Chapter 1 What Is Different from Pathway Simulation?

As described in Introduction, cell simulation employs modeling of 3D space in addition to pathway diagram enabling simulating localized protein activation and its spatial spreads. This is the difference between cell simulation and pathway simulation. More specifically, cell simulation enables us spatial signal transduction starting at receptors on the plasma membrane to the nucleus (Figure 1). To realize this, we should embed different reactions at different cellular sub-regions as well as transmit the signal of protein activation to spatially distant location. Thus proteins should 'move' within a 3D space[3]. Thus the essential difference is that in 4D cell simulation movement/translocation of proteins are involved, which is neglected in pathway simulation.

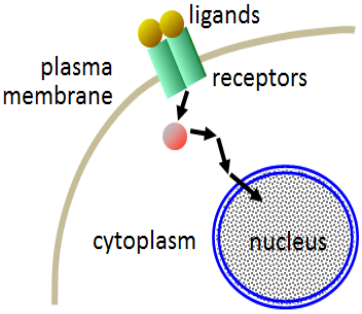


Figure 1 Spatio-temporal signal transduction.

There are three potential mechanisms of movements to be considered: diffusion, transportation, and mobility[4]. Diffusion is a random movement of molecules by thermal energy in microscopic view, but it is a gradual spreading of an ink droplet in water in macroscopic view. Transportation is the active transport of proteins by motor protein such as kinesin. Mobility is a movement by a gradient of electrical potential. Among them, diffusion is described in this eBooklet because it is the basis for cell simulation.

There might be no comprehensive lecture on diffusion in the present molecular cell biology. However, diffusion is the most important mechanism by which signal is transmitted to the distant location in a cell. Thus, diffusion is essential in 4D cell simulation. Spatial difference in the concentration is the driving force of diffusion, and Fick's first low describes the flux by diffusion $J_{diff}$ (mol/m$^2$/s), which is the amount of a substance flowing through a unit area in a unit time.

$$J_{diff} = -D\frac{\partial C}{\partial x} \qquad 1)$$

where D is the diffusion coefficient (m$^2$/s), and $\partial C/\partial x$ is the spatial gradient of concentration (mol/m$^4$). When concentrations at two positions with distant $\Delta x$ are $C_1$ and $C_2$ (Figure 2), discretized spatial gradient is as follows:

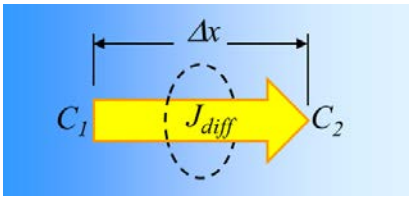


Figure 2 Flux by diffusion.

$$spatial\ gradient = \frac{C_2 - C_1}{\Delta x} \qquad 2)$$

This indicates that larger the concentration difference and smaller the spatial distance, flux $J_{diff}$ is

[3] It is thought that electromagnetic field is not important in the signal transduction in a cell except voltage-sensitive channels. This view leads to an important consequence that movement of proteins is essential for signal transduction, because direct binding between proteins is required for the signal transduction.

[4] While pressure can cause the movement of molecules, its effect is negligible in a cell.

larger. *D* is the constant of the proportionality, which ranges $10^{-12}$～$10^{-10}$m$^2$/s for most proteins.

Equation 1) provides us an important equation that gives us a measure of spatial spread λ (m) of molecules at time t after the start of diffusion, where molecules were confined at *x=0* at *t=0*.

$$\lambda^2 = 2Dt \quad \text{1D}$$
$$\lambda^2 = 4Dt \quad \text{2D} \qquad \text{3)}$$
$$\lambda^2 = 6Dt \quad \text{3D}$$

Note that the equation depends on the spatial dimension. If *D* of a protein is $10^{-11}$m$^2$/s, it will reach 14.5 μm ($=\sqrt{2 \cdot 10^{-11} \cdot 10}$) from the origin 10 s after the start of diffusion in 1D diffusion. Equation 3) is frequently used in the validation and analyses of 4D cell simulation results.

Equation 3) for 1D is derived as follows. (This note can be skipped.) Let's start at continuity equation, which describes local mass conservation as follows:

$$\frac{\partial C}{\partial t} = -\frac{\partial J_{diff}}{\partial x} \qquad \text{A1)}$$

This indicates that the change in the amount in a volume of interest is equal to the amount flowing into or out of a volume indicating that there is neither generation nor sink of molecules in a volume. On the other hand, we get Equation A2) by differentiating Equation 1) with respect to *x*.

$$\frac{J_{diff}}{\partial x} = -D\frac{\partial^2 C}{\partial x^2} \qquad \text{A2)}$$

By substituting A2) into A1), we get famous diffusion equation as follows:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \text{A3)}$$

Integrating Equation A3) by the boundary conditions where *C=1 at x=0, C=0 at x>0*, and $\int_{-\infty}^{+\infty} C \cdot dx = 1$ at t=0 yields a solution for 1D diffusion equation.

$$\mathrm{C} = \frac{1}{\sqrt{4\pi Dt}}\exp\left(-\frac{x^2}{4Dt}\right) \qquad \text{A4)}$$

On the other hand, Gaussian distribution with mean and standard deviation of μ and σ$^2$, respectively is as follows:

$$f = \frac{1}{\sqrt{2\pi\sigma^2}}\exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) \qquad \text{A5)}$$

By comparing Equations A4) and A5), A4) is found to be a Gaussian distribution with μ=0 and $2\sigma^2 = 4Dt$ yielding $\sigma^2 = 2Dt$. σ is a measure of a spread, and hence λ gives us a measure of spatial spread of diffusing molecules.

# Chapter 2 Inside Cell Simulation

In Chapter 1, we have described that (1) cell simulation is different from pathway simulation in that 'movement' of proteins is simulated, (2) diffusion is the basic mechanism for 'movement' of proteins, and (3) Fick's first law is used for calculating diffusion. These are conceptual bases and basic principles for cell simulation. In this chapter, we describe how cell simulation is realized from these bases.

First, we need to manage 3D space. Commonly employed method to manage 3D space is to divide a 3D shape into small volumes[5]. We call one small volume a compartment. This method enables us to divide a given shape into many compartments filling the inside of the shape. In addition, localized reactions in a shape are realized by embedding different reactions to localized compartments. The same reactions are assumed to proceed simultaneously and homogeneously within a single compartment[6]. Diffusion is approximated by calculating $J_{diff}$ between adjacent compartments using Fick's first law (Figure 3)[7]. Thus, reaction and diffusion are coupled together in 4D cell simulation. These are mechanisms inside the cell simulation.

Next, we discuss how reactions and diffusions are coupled into a single differential equation with respect to each protein in a compartment. Reactions can be converted into differential equations as discussed in an eBooklet "Learn Pathway Simulation in 10 pages". Fick's first law is also a differential equation. Therefore these two types of differential equations are linearly combined as follows:

$$\frac{\partial C}{\partial t} = \left(\frac{\partial C}{\partial t}\right)_R + \left(\frac{\partial C}{\partial t}\right)_D \qquad 4)$$

Equation 4) is called a reaction-diffusion equation, and the first and the second terms on the right are reaction and diffusion terms, respectively. An example for 1D with three compartments is shown in Figure 4. If we focus on the middle compartment, it has only two adjacent compartments. Let cross sectional area between compartments be S, and distance between centers of two compartments be L. In this example, it is assumed that the same reaction is embedded to all compartments but with different concentrations.

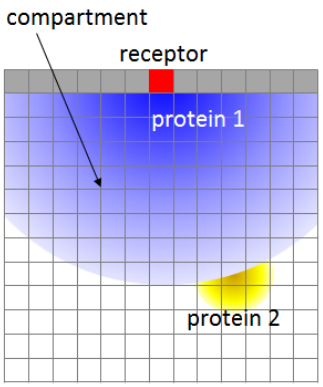


Figure 3 Activation spread in a discretizing shape into compartments. In this 2D example, protein 1 (blue) is activated at a receptor compartment (red) and begins to diffuse into all over the shape activating protein 2 (yellow). Compartments with cell membrane are shown in gray.

5 Several methods of division can be applied including triangles or quadrilateral for 2D and tetrahedrons or hexahedrons for 3D shapes. In this eBooklet division by quadrilateral for 2D and hexahedrons for 3D is assumed. There might be some problems for these divisions, however, such problems can be negligible and/or avoidable in cell simulation.

6 If the size of compartments is small, this assumption will be valid.

7 We cannot avoid errors in the calculation of diffusion since the size of the compartment is larger than zero. This is a volume discretizing error, which can be compared to the error arising from the non-zero calculation time step in pathway simulation. We should compromise between errors and realistic simulation time in running simulation.

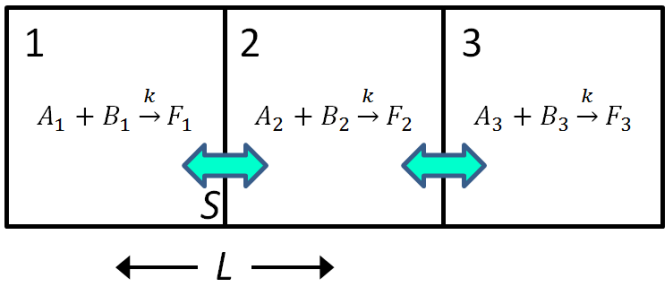


Figure 4 Basic mechanism of reaction-diffusion simulation.

If we consider the center compartment (compartment 2), the differential equation for reaction of molecule F is described in a discretized form as follows:

$$\left(\frac{\Delta F_2}{\Delta t}\right)_R = k \cdot A_2 \cdot B_2$$

The differential equation between adjacent two compartments is discretized in a discretized form as follows:

$$\left(\frac{\Delta F_2}{\Delta t}\right)_D = D_F \frac{F_1 - F_2}{L} S + D_F \frac{F_3 - F_2}{L} S$$

Thus we obtain discretized reaction-diffusion equation by combining these two equations.

$$\frac{\Delta F_2}{\Delta t} = k \cdot A_2 \cdot B_2 + D_{F_2} \frac{F_1 - F_2}{L} S + D_{F_2} \frac{F_3 - F_2}{L} S \qquad 5)$$

This is the discretized reaction-diffusion equation for molecule F in compartment 2. If there are other reactions with respect to F, additional reaction terms are included. If the shape is 2D or 3D, additional diffusion terms between adjacent compartments are included because the number of adjacent compartments is increased to 4 or 6. Equations for compartments 1 and 3 are derived by using $A_1$, $B_1$, $F_1$ and etc. instead of $A_2$, $B_2$, $F_2$, which can be easily derived by referring Equation 5). By preparing Equation 5) for all molecules and for all compartments, we can run simulation by the similar methods as described in an eBooklet "Learn Pathway Simulation in 10 pages".

As shown in this chapter, equations to be calculated in cell simulation are not complex, because it is a linear combination of equations for reaction and diffusion. However, we need to write 10,000 equations of for each molecule if there are 10,000 compartments. In addition, we should write them carefully without mistakes in the connections between adjacent compartments. This is time consuming and almost unrealistic when the shape is complicated and divisions are large. Thus we need specialized software. A-Cell is unique software for this purpose. A-Cell generates shapes that are divided into compartments, and also generates simulation programs with connecting information between compartments in a shape. Thus A-Cell makes 4D cell simulation realistic.

# Chapter 3 Why Do We Need Cell Simulation?

We described in Introduction that the final goal of simulation in molecular cell biology is the whole cell simulation, and hence, simulation should be spatio-temporal. In this chapter, we show four essential pints that cell simulation can manage but pathway simulation cannot, indicating critical importance of cell simulation.

**1) Investigating roles of shape of a cell and its intracellular structure:** Each cell possesses unique shape and intracellular structure. Cell changes its shape and structure according to microenvironment surrounding it. Cell simulation can investigate these effects, but pathway simulation cannot.

**2) Discrimination of membrane (2D) and cytoplasmic (3D) reactions:** There are so many membrane reactions in addition to bulky cytoplasmic (aqueous) reactions. Proteins shuttle between the cytoplasm and the membrane by posttranslational modifications. Then, the question is what the difference is in terms of reaction speed[8]. Figure 5A shows spreading of molecules in 1D, 2D and 3D space after $4t_0$ starting at $t_0$. By the increase in the spatial dimension, molecular spreading is larger, and the concentration at the center is lower. This indicates reaction in 2D is more advantageous than 3D because molecules are more crowded for a longer period of time in 2D.

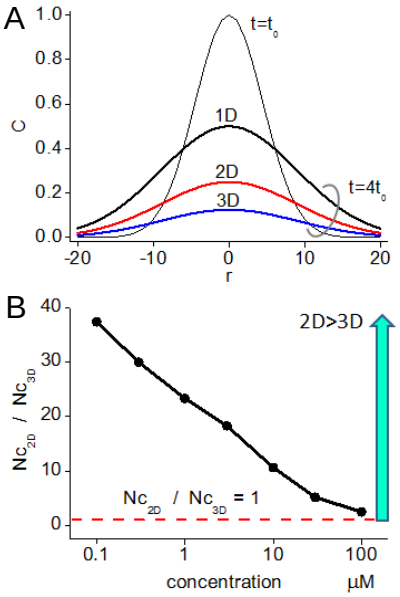


Figure 5 A comparison between membranous (2D) and cytoplasmic (3D) reactions.

Next we compare number of collisions in unit time in 2D and 3D with the same average inter-molecular distance and diffusion coefficient using stochastic particle simulation (Figure 5B)[9]. Horizontal axis is the concentration in 3D. If the ratio $NC_{2D}/NC_{3D}$ is larger than 1, the number of collisions in 2D is larger than 3D, and hence the reaction is expected to proceed faster in 2D. As shown in Figure 5B, the ratio is larger than 1 at concentration below 100 μM[10]. Thus translocation of proteins between the cytoplasm and the membrane can regulate the reaction. This regulation cannot be simulated without 4D cell Simulation[11].

**3) Importance of amount as well as concentration:** Concentration is a major variable in pathway simulation. However, the amount is also an important variable. This importance is shown here by an example in an eBooklet "Learn Utilization of Cell Simulation in 10 pages". MT1-MMP is a membrane type ECM degrading protein. MT1-MMP is inhibited endogenous soluble inhibitor

8 This and related topics have a long history. Cf. McCloskey, M.A., et al., JBC., 102 (1986) 88; Orr, G., et al., Biophys.J., 89 (2005), 1362; Knight, J.D., Biophys.J., 99 (2010), 2849; Weiß, K., et al., Biophys.J., 105 (2013), 455; Boggara, M., et al., BBA, 1828 (2013), 419.

9 Ichikawa, K., et al., Physical Biol., 7 (2010), 046010.

10 The difference in diffusion coefficient in 2D and 3D possibly changes the result. However, 2D reactions might provide another advantageous mechanism, in which diffusion barriers by cortical actin mesh can restrict the area of molecular diffusion (pickets model).

11 Translocation of proteins between membrane and the cytoplasm and 2D and 3D diffusion can be simulated using A-Cell.

TIMP-2. Pathway simulation replicated the ECM degradation observed in vitro (red line in Figure 6). In experiments, MT1-MMP expressed at a confined region on the membrane (orange puncta in the inset of Figure 6). Then we ran 4D simulation for a region indicated by a white square. MT1-MMP was assumed to be highly expressed at a red circular region. TIMP-2 diffused in all regions of the shape. It was surprising to find that almost no degradation of ECM was seen by 4D simulation with the same parameter values as those of pathway simulation (blue line in Figure 6)[12]. Then we ran simulation by stopping diffusion of TIMP-2. This leads to an almost identical result as pathway simulation (pink line in Figure 6), indicating that in 4D simulation, TIMP-2 is supplied to the region of MT1-MMP by diffusion, by which MT1-MMP was fully suppressed[13]. Thus, the amount of TIMP-2 plays an important role, which can be properly simulated by 4D simulation but not by pathway simulation.

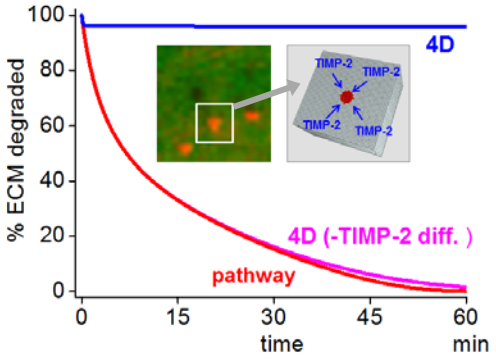


Figure 6 Comparison of 4D cell and pathway simulation in ECM degradation by MT1-MMP.

**4) Pattern formation:** As described in Introduction, localization of proteins is frequently observed in a cell. Here we call pattern when a system is neither random nor uniform. Patterns can emerge in 4D cell simulation from random or uniform distribution of molecules. Figure 7 shows an example of 4D cell simulation for actin regulating proteins, which is important to cell migration[14]. A small G protein Cdc42, Rac and Rho play differential roles in regulating F-actin dynamics (Figure 7A). Simulation results showed distinct spatial distributions for these proteins (Figure 7B), which were thought important for cell migration and/or change in the shape. These were never obtained by pathway simulation.

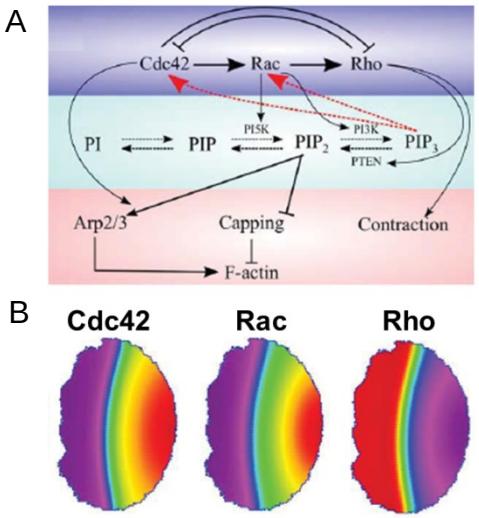


Figure 7 4D cell simulation for proteins regulating actin dynamics[12]. Different colors indicate intracellular distribution of proteins.

We have shown four essential points that are managed by 4D cell simulation but not by pathway simulation. These indicate that 4D cell simulation is essential to simulate functions and mechanisms of a cell, and suggest that it is a powerful tool in revealing cellular mechanisms.

12 Hoshino, D., et al., PLoS Comp.Biol., 8(2012), e1002479; Watanabe, A., et al., PLoS Comp.Biol., 9(2013), e1003086.
13 Parameter values were found in published papers
14 Maree, A.F.M., PLoS Comp. Biol., 8(2012), e1002402.

# Chapter 4 An Example of Cell Simulation by A-Cell

In this chapter an example of cell simulation is shown aimed at illustrating its importance. Cell simulation can provide us new findings that are not reached by pathway simulation[15]. NF-κB is a transcription factor activated by cytokines such as TNF-α. Upon activation of NF-κB, it translocate from the cytoplasm to the nucleus, where it regulates gene expression profile (Figure 8). Its signaling cascades are as follows:

TNF-α receptor (plasma membrane) → NF-κB activation (cytoplasm) → gene expression (nucleus)

Thus this signal transduction covers from the plasma membrane to the nucleus. Importantly the expression of IκB gene, which generates NF-κB inhibitory protein IκB, is enhanced by NF-κB. Thus a negative feedback loop emerges (Figure 8), leading to the repetitive translocation of NF-κB between the cytoplasm and the nucleus and generating oscillation of nuclear NF-κB.

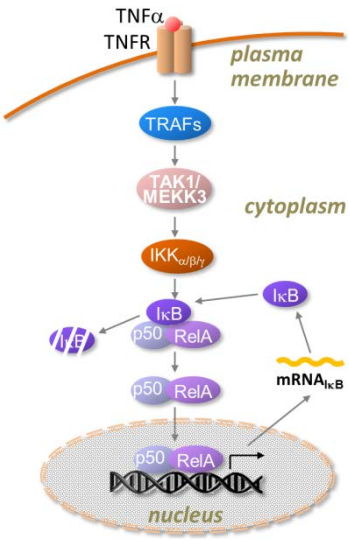


Figure 8 NF-κB signal transduction.

Although there are more than 60 simulation papers published[16], no 4D cell simulation was reported. We wanted to investigate the effect of spatial parameters on the oscillation of NF-κB by constructing spherical cell model with the diameter of 50 μm, and compared 4D cell simulation with pathway simulation (Figure 9). Reactions such as activation of NF-κB in the cytoplasm, translocation via nuclear membrane, and transcription in the nucleus were embedded to the corresponding sub-regions in the spherical cell model, and all proteins and mRNA were set to be diffused (Figure 9A). First we ran pathway simulation (red curve), and compared it with experiment (black dots), showing a good agreement with oscillation period of about 1.5 hrs (upper panel of Figure 9B)[17]. Next we ran 4D cell simulation with the same values in all parameters. Surprisingly, however, the oscillation period was considerably prolonged to 5 hrs (lower panel in Figure 9B). This clearly indicated that the space had significant effect on the oscillation pattern of NF-κB.

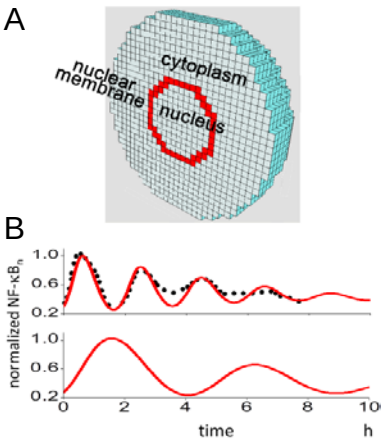


Figure 9 Spherical cell model for NF-κB simulation (A) and comparison between pathway simulation (upper) and cell simulation (lower).

Then we investigated how each spatial parameter affected the oscillation pattern. We tested five parameters including 1) diffusion coefficient, 2) nuclear to

15 Ohshima, D., et al., PLoS ONE, 10 (2015), e0127633; Ohshima, D., et al., PLoS ONE, 9 (2014), e109895; PLoS ONE, 7 (2012), e46911.

16 Ichikawa, K. et al., IET Systems Biology, 9 (2014), 41.

17 Sung, M.H., et al, PLoS ONE, 4 (2009), e7163.

cytoplasmic volume ratio (N/C ratio), 3) transport via nuclear envelope, 4) cytoplasmic loci of IκB protein synthesis, and 5) shape of the nucleus. Although the diffusion coefficient is inherent to each protein, its effective value can be altered because of the crowding of mitochondria around the nucleus due to hypoxic condition[18]. N/C ratio was reported to be altered by the increasing malignancy of cancer cells[19]. Transport via nuclear envelope was reported to be altered because of the senescence[20]. The shape of the nucleus was also reported to be altered in some diseases and by age. The location of translation is not known. On the other hand, it was suggested that the oscillation pattern of nuclear NF-κB affected the gene expression profile[21]. Thus investigating the effect of these spatial parameters was biologically important. However, experimental alteration of these parameters was not easy. Thus we investigated these by simulation because any parameters in simulation can be changed. After changing non-spatial parameters so as to get the same oscillation pattern as experiments, we ran 4D cell simulation by changing spatial parameters.

Summary of simulation results are shown in Figure 10. By increasing (red curve in Figure 10A) or decreasing (blue curve) N/C ratio, diffusion coefficient, and transport via nuclear envelope within a biologically relevant range, oscillation pattern, which is measured by frequency and persistency, was considerably changed from the control (black curve). The location of IκB protein translation also largely changed the oscillation pattern. While there was virtually no change by the change in the nuclear shape from a sphere to a cube keeping N/C ratio unchanged. Next we performed a detail analyses and found that translocation of cytoplasmic IκB to the nucleus specifically regulated frequency, while diffusion coefficient of mRNA and IκB, and transport of IκB mRNA from the nucleus to the cytoplasm specifically regulated persistency.

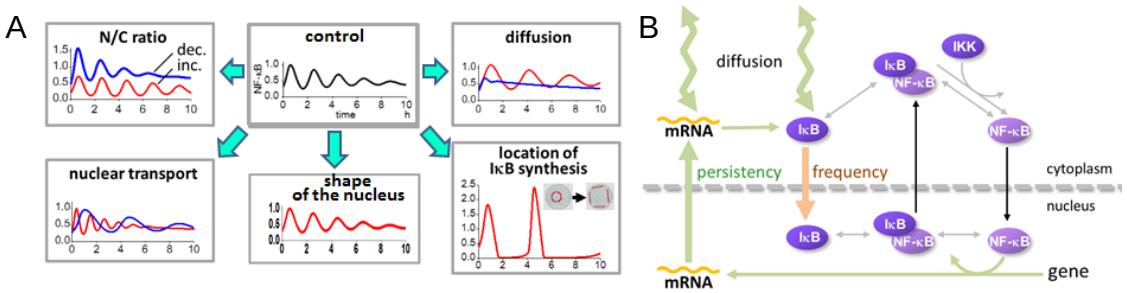


Figure 10 Spatial parameters changed the oscillation pattern of nuclear NF-κB.

Thus it was strongly suggested that spatial parameters regulated the oscillation pattern of NF-κB, and thus the gene expression profile. We can reach to these results by 4D cell simulation but not by pathway simulation. Thus 4D cell simulation is important to reveal the nature of the biological phenomena and their mechanisms.

18 Al-Mehdi, A.B., et al., Sci.Signal., 5 (2012.), ra47.
19 Pienta, K.J., et. al., Cancer, 68 (1991), 2012.
20 Kim, S.Y., et al., BBRC, 391 (2010), 28.
21 Ashall, L., et al., Science, 324 (2009), 242.

# Summary

In previous chapters, we have first described the principle of 4D cell simulation, and next we have shown that the difference between 4D cell and pathway simulation existed in that 4D cell simulation included movement of molecules such as diffusion. We have also shown that special software is required because many repetitive descriptions are required for running 4D cell simulation. It is evident that 4D cell simulation is totally different from pathway simulation in which different names are used for the cytoplasmic and the nuclear species to discriminate protein location by name but not by spatial location. 4D cell simulation is also different from two compartment simulation, in which there are compartments for the cytoplasm and the nucleus for example, because this simulation cannot investigate the location of translation as described in Chapter 4. Storing IκB in the cytoplasm is essential for persistent oscillation of NF-κB, which was not discussed in Chapter3. In this case, the cytoplasm near and distant from the nucleus plays a different role. Two compartments simulation cannot distinguish this. Thus 4D cell simulations are essential for elucidating true mechanisms in a cell. We do not know the entire mechanisms for cellular functions. In this situation, attentive simulation is important, and would lead to reliable findings.

To close this eBooklet, we introduce one of our experiences. We presented localization of proteins within a tiny protrusion of which size was less than 1 μm, and it persisted more than 1 s. One researcher commented that he did not agree with our result, because a protein spread within 0.2 s according to Eq. 3) if D was $10^{-11}m^2/s$. This was a good point, however, an important condition for applying Eq. 3) was missed. Eq. 3) assumes that all molecules are confined at a single point at t=0, and there is no molecular source. In our simulation, however, diffusing protein were activated by extracellular ligand, and activation persisted as long as the ligand existed, which served as a source for diffusion. Thus Eq. 3) was not applicable in our case. Figure 11 illustrates this difference. Upper two panels show molecular distribution by diffusion without any source. Molecules are almost homogeneously distributed at time t. Lower two panels show diffusion with a source at the center. In this case, prominent molecular localization remains even at 66.7 times longer than the upper case. White lines indicate the spatial distribution along diameters. Eq. 3) is useful, but we should be careful in its application.

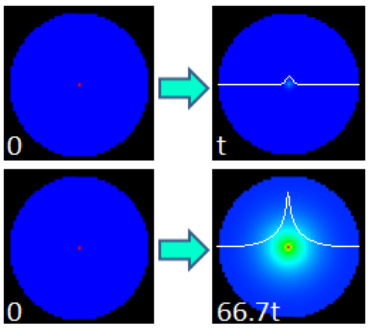

Figure 11 Simulations of diffusion with (lower) and without a source (upper). In case without a source, molecules spread quickly. In case with a source, however, inhomogeneous distribution persisted for a much longer period of time. See Simple Diffusion and Source Diffusion in A-Cell models.

We described the bases of 4D cell simulation, its mechanisms, and its importance. 4D cell simulation is a powerful tool for elucidating cellular mechanisms, and we hope that it will be popular in the simulation of molecular cell biology.