Pathway Simulation by A-Cell

ERK Signal Transduction

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Kazuhisa Ichikawa

True Cell Simulations Inc.
http:// engl.tc-simulations.com/

True Cell Simulations Inc. Tokyo Japan

Contents

About This eBooklet

Two ebooklets, “Learn Pathway Simulation in 10 Pages” and “Learn Utilization of Simulation in 10 Pages” were published on our website aimed at introducing pathway simulation and its utilization. “Learn Pathway Simulation in 10 Pages” describes basic principles and mechanisms of pathway simulation. “Learn Utilization of Simulation in 10 Pages” describes how simulation is applied in experimental research showing eight examples in four categories. These two eBooklets are useful to learn bases of pathway simulation and its utilization. It has not been described, however, how researches can run simulations specific to their experimental research. Obviously, general description on this topic is not easy, because every experimental research is focusing on specific pathways and roles of proteins that have not been reported before. Thus, researchers should construct novel pathway specific to their own experimental results.

However, every experimental research has preceding studies by other laboratories. If a pathway model was reported in a previous report, researchers can modify it for their specific use by adding, deleting, and/or changing interactions between proteins. This allows researchers to run simulations much easier than creating a pathway from scratch.

The first step in this approach is to reconstruct a published simulation. However, there are two problems. First, it is not easy to understand published simulation papers for reconstruction, especially in case where a model is described mathematically. Some equations may be missing, and one should add missing equations. Second, even if one can understand a simulation paper enough for reconstructing it, one might have a trouble in how one can do it, i.e. what software can be used.

If reported simulations are reconstructed by A-Cell, the second problem shown above is solved. Researchers can modify reconstructed A-Cell model for their own purpose and can start their simulation at this reconstructed A-Cell model. Thus, we should solve the first problem.

The purpose of this eBooklet is to reconstruct simulation in a published paper. A-Cell model files are provided and downloadable freely, which enables running a sample simulation. If one modifies the initial conditions, one can run simulations with different parameter sets, i.e. with different rate constants and/or concentrations. One can modify downloaded A-Cell models specific to his/her purpose.

Many simulation papers have been published on different topics. So we are planning to publish several eBooklets on the reconstruction by A-Cell providing A-Cell model files for above use. We hope that you find relevant A-Cell models for your own research. This is the first eBooklet, where we have reconstructed ERK signaling pathway originally published by Shankaran, et al [8].

This eBooklet also provides information to construct a pathway model from scratch. Through processes and methods for the reconstruction described in this eBooklet, one can learn how to create one’s own original model too.

1. ERK Signal Transduction

Figure 1 illustrates pathways focusing on cell proliferation and apoptosis appeared in Wikipedia on the net [1]. Dysregulation in proliferation and apoptosis are closely related to carcinogenesis and malignant tumors, and have been receiving great attention by many researchers. MAPK family proteins are involved in these pathways (gray area in Figure 1). Among MAPK family proteins, ERK is the first protein identified in this pathway (blue rectangle in Figure 1), where epidermal growth factor receptor (EGFR) is activated by EGF triggering activations of downstream proteins leading to the phosphorylation of ERK, which is the activated form of ERK (Cf. the following scheme).

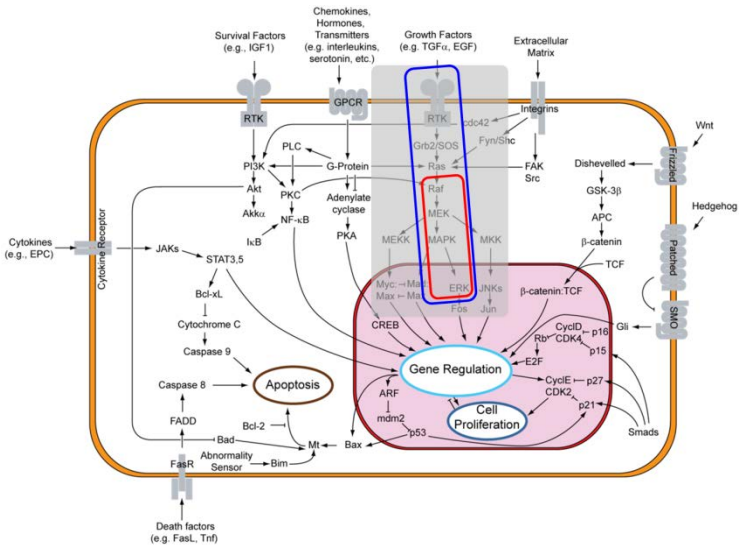

Figure 1 A pathway focusing on cell proliferations and apoptosis.

$$\text{EGFR} \rightarrow \text{Ras} \rightarrow \text{Raf (MAPKKK)} \rightarrow \text{MEK (MAPKK)} \rightarrow \text{ERK} \qquad 1)$$

Activated ERK activates transcription factors in the nucleus leading to the regulation of gene expression profiles.

Ras in Eq.1) is activated by GDP-GTP exchange activity by SOS, which is triggered by active EGFR. Interestingly, it was reported that active ERK deactivated SOS, leading to the deactivation of Ras [2]-[3]. Thus, activated ERK is deactivated again. Figure 2 shows a detail ERK pathway including this negative feedback, which was used in an eBooklet “Learn Utilization of Simulation in 10 Pages”. Here, Ras is ommited, and pathway for activating MAPKKK (MKKK) by EGFR is simplified. Negative feedback pathway in which SOS is deactivated by active ERK leading to the deactivation of Ras is also simplified by a line designated by “negative feedback”. In some pathway models, only reactions in focus are modeled in detail (red rectangle in Figure 1, in this case). There will be discussions for this simplification. This will be discussed elsewhere.

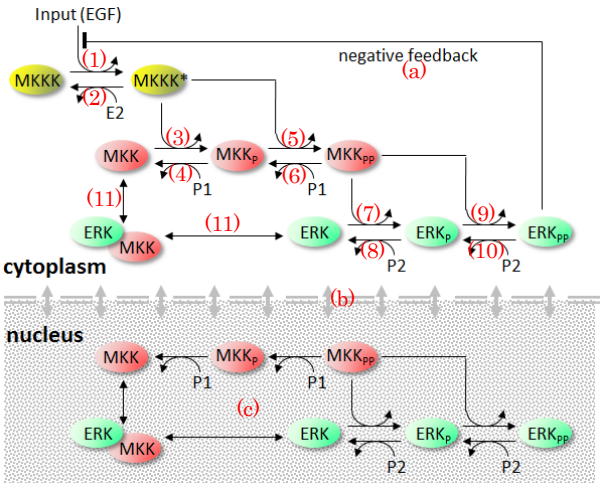

Figure 2 ERK pathway.

2. A model for ERK Signal Transduction

Many pathway simulations on ERK signaling have been reported. Some of them are listed in the Reference [4]-[8]. Among them, a pathway shown in Figure 2 was derived from a paper by Shankaran, et al., 2009 [8]. In their paper, ERK-GFP fusion protein was expressed in cells, and nuclear ERK oscillation, that is the repetitive up and down of nuclear ERK fluorescence intensity, was measured in single cells. Stimulation by EGF resulted in the oscillation of total nuclear ERK intensity, which was the summation of the fluorescence from ERK, ERKp, and ERKpp in the nucleus, and in phosphorylated nuclear ERK, which was the summation of ERKp and ERKpp in the nucleus, with repetitive time interval of 15 min (Figure 1C in Shankaran's paper [8]). They constructed a pathway model and ran simulations comparing results with their experiments. In this eBooklet, we reconstruct a simulation in Shankaran's paper by A-Cell.

Before the reconstruction by A-Cell, reactions shown by Eq.1 in Supplementary Information of Shankaran's paper were redrawn by A-Cell (Figure 3).

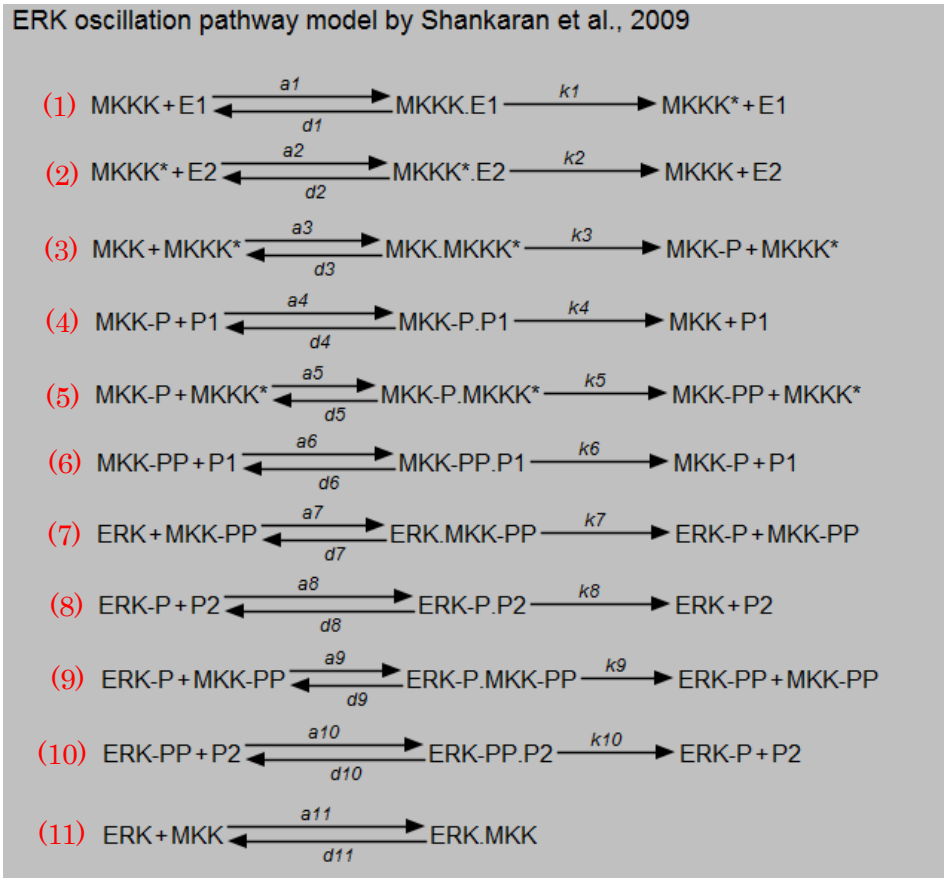

Figure 3 Reactions in Shankaran's model drawn by A-Cell.

First we should confirm that reactions shown in Figure 3 correctly represent the pathway shown in Figure 2. It is important to notice that activation and phosphorylation reactions are assumed to be enzymatic reactions. One can know this by looking into the pathway shown in Figure 2 carefully. So

reactions are drawn as Michaelis-Menten enzymatic reactions in Figure 3 except the reaction between ERK and MKK binding and dissociation. Let's investigate corresponding reactions in Figures 2 and 3. To make this easier, we added line number (red numbers in parentheses of Figure 3).

It is obvious that reactions in line (1) of Figure 3 correspond to the reaction (1) in Figure 2. There are 4 species, MKKK, E1, MKKK.E1, and MKKK* in line (1) of Figure 3, however, only two of them are drawn in Figure 2 (MKKK and MKKK* shown by yellow ellipses), and remaining two (E1 and MKKK.E1) are missing. MKKK.E1 is an intermediate complex in the enzymatic reaction, and is omitted in the reaction (1) of Figure 2 for simplicity. E1 in line (1) of Figure 3 acts as an enzyme converting MKKK to its active form MKKK*. Therefore, it is easily recognized that E1 corresponds to Input (EGF) in Figure 2. Thus line (1) in figure 3 corresponds to reaction (1) in Figure 2. In the same way, we can confirm that reactions in lines (2)-(11) correspond to reactions (2)-(11) in Figure 2. Thus, all reactions in Figure 3 exist in Figure 2.

However, negative feedback reaction (a), translocation of proteins between the cytoplasm and the nucleus (b), and reactions in the nucleus are not shown in Figure 3. Among them, it was clearly described in Shankaran's paper that the nuclear reactions were included in the simulation with the same schemes and rate constants. Thus we should make clear how negative feedback and nuclear transport were realized in their simulation.

Among them, nuclear transport was not shown in a form of reaction schemes. Instead, it was shown by a set of differential equations (Eq.2 in Supplementary Information by Shankaran's paper). According to their equations, the changes in the concentration of i[th] proteins in the cytoplasm (x_i^c) and the nucleus (x_i^n) were calculated as follows:

$$\frac{dx_i^c}{dt} = b_i^c - t_i^{imp} \cdot x_i^c + \frac{t_i^{exp}}{V} \cdot x_i^n \qquad 2)$$

$$\frac{dx_i^n}{dt} = b_i^n + t_i^{imp} \cdot \mathrm{V} \cdot x_i^c - t_i^{exp} \cdot x_i^n \qquad 3)$$

b_i^c and b_i^n designate concentration changes by reactions, and t_i^{imp} and t_i^{exp} are rates of transport through the nuclear envelope to and from the nucleus, where V is the reciprocal of nuclear to cytoplasmic volume (N/C) ratio. The nuclear transports are shown in the second and the third terms on the right sides in Eqs.2) and 3). It should be noted that t_i^{exp} in Eq.2) is divided by V, which leads to the smaller rate of the change in x_i^c because V=6 in Shankaran's paper. Thus, smaller cytoplasmic change in the concentration by the export of proteins from the nucleus of small volume was realized. The second term in the right side in Eq.3) was multiplied by V realizing larger change in the concentration of the nucleus by larger volume of the cytoplasm than the nucleus.

Thus it is clear why Shankaran et al. didn't use reactions for the nuclear transport. To realize the effect of smaller nuclear volume than the cytoplasm in the simulation, the rate constant for the same

species should be different for the change in cytoplasmic and nuclear concentrations ($\frac{t_i^{exp}}{V} \cdot x_i^n$ in Eq.2) and $t_i^{exp} \cdot x_i^n$ in Eq.3)). These modifications were required to realize volumetric effect in the pathway simulation that does not include volumetric dimension (i.e. spatial dimension). If you run 4D simulation by A-Cell, however, these modifications are not required[1].

Lastly, we should know how negative feedback was realized in Shankaran's paper for reconstruction. It was written in Supplementary Information that the following equation was used[2]:

$$E1'_{tot} = \frac{E1_{tot}}{1+[ERK-PP]^c/K_i} \qquad 4)$$

This indicates that $E1'_{tot}$ was used instead of E1 in the simulation. Since there is one variable *[ERK-PP]* on the right side of Eq.4), $E1'_{tot}$ depends only on *[ERK-PP]*. This dependency is determined by 3 constant parameters, $E1_{tot}$, *C, and* K_i. To show the dependency, Eq.4) is graphically shown in Figure 4. Black curve is a control curve, where $E1'_{tot}$ becomes smaller by the increase in *[ERK-PP]*. This relationship is easily estimated from Eq.4) by assigning *[ERK-PP]=0 and [ERK-PP]=*∞, because these give $E1'_{tot} = E1_{tot}$ and $E1'_{tot} = 0$, respectively. When K_i is larger, the curve shifts to the right (a green arrow and a continuous gray curve), because larger *[ERK-PP]* requires for the same decreased value in $E1'_{tot}$. When *C* is larger, the steepness of the curve increases (blue arrows and a broken gray curve), because the effect in the increase or decrease in *[ERK-PP]* becomes larger. In summary, increase in *[ERK-PP]* leads to the decrease in $E1'_{tot}$ and in its potential to activate MKKK, thus realizing the negative feedback by active ERK.

It should be better to construct a reaction model for negative feedback using SOS, Ras, and ERK. However, a part of a model is often made simpler as shown here, if it is not essential in the simulation. This is the same concept as the simplification discussed in Chapter 1. The validity for such simplification will be discussed elsewhere. Finally it is worth noting that Eq.4) and its graphical representation in Figure 4 are recommended to be memorized, since Eq.4) and its modified form is often appears in simulation papers.

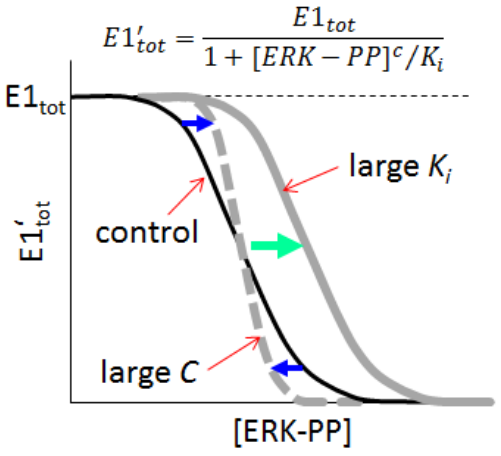

Figure 4 Graphical representation of Eq. 4)

[1] This eBooklet focuses on pathway simulation without spatial dimension. 4D simulation will be shown in different eBooklet.

[2] Eq.4) was shown in a text of Supplementary Information in Shankaran's paper. Although the negative feedback was critical for their simulations, its model was not shown as clear as in Eqs.2) and 3), and careful reading of the manuscript is required. This might be one reason for difficulties in understanding the model, because different expressions of the model were used in the same manuscript, and combining all these different expressions were required for the reconstruction of the simulation. This seems common to most of the simulation papers.

3. Concentrations, Rate Constants and Other Parameters

Setting parameter values for simulation is important because they can change the behavior of the system dramatically. If a simulation was run with biologically irrelevant parameter values, the results could also be biologically irrelevant. All parameter values should be listed in a paper. In the paper by Shankaran et al. [8], parameters are listed in Table S1 in the Supplementary Information. This table summarizes rate constants including nuclear transport, and concentrations of all proteins. However, rate constants a_7, a_9, and a_{11} were not shown in Table S1. Instead A_7, A_9, A_{11}, K_{s7}, K_{s9}, and K_{s11} were shown, because the complex formation steps in these reactions were assumed to saturate out as the substrate concentration was increased. In other studies, quasi-equilibrium between enzyme and substrate were assumed as was seen in textbooks of enzymatic reactions [4], [5]. We believe that these assumptions were dangerous in studying dynamics of signal transduction, because it is not always true that the assumption of quasi-equilibrium holds. This assumption holds only within a certain range of rate constants as shown in textbooks of enzymatic reaction. In the analyses of dynamic systems, however, rate constants are changed possibly beyond the range assuring the quasi-equilibrium assumption. Therefore, in this reconstruction by A-Cell, a_7, a_9, a_{11}, d_7, d_9, d_{11}, k_7, k_9, k_{11} are used without assuming any quasi-equilibrium. Among them, d_7, d_9, d_{11}, k_7, k_9, k_{11} are listed in Shankaran's Table S1. a_7, a_9, a_{11} are listed in separate Supplementary Information in an Excel file, and values designated by "Current study (Base set)-P_o" were used.

Next we should find values for C and K_i in Eq.4). Although K_i was shown in Table S1, C was not shown. So we assumed the most simple case, where $C=1$.

Finally we describe initial protein concentrations. As described above, concentrations were listed in Shankaran's Table S1, but in total concentrations. However, MKK, ERK, and ERK.MKK complex are distributed both in the cytoplasm and in the nucleus in the absence of ERK activation. Therefore, we should find values for these species both in the cytoplasm and in the nucleus before starting simulations. In general, however, it is difficult or even impossible to calculate these values analytically. In such case, a simulation acquiring concentrations at equilibrium is run. We employed this method here assigning each total concentration to cytoplasmic MKKK, MKK, and ERK setting zero values to all other species as an initial condition. After reaching to an equilibrium state, initial concentrations were redistributed to corresponding cytoplasmic and nuclear values. These values were used for the simulation. It is important to assure that the system is in an equilibrium state before starting any simulation.

Parameter values in a simulation should carefully be defined as described above. Finding parameter values is time-consuming, but is important. A list of parameter values for A-Cell simulation is listed in the following page.

4. ERK Pathway Model by A-Cell

In this Chapter we construct A-Cell model of ERK signaling by Shankaran, et al.[8]. Figure 3 was one form of A-Cell reconstruction. It was a replication of Shankaran's model expression. This expression is suitable for showing reaction kinetics, which was Michaelis-Menten type kinetics. However, this expression was not suitable for showing the structure of the signaling pathway. Therefore, Shankran's model was reconstructed differently by A-Cell as shown in Figure 5.

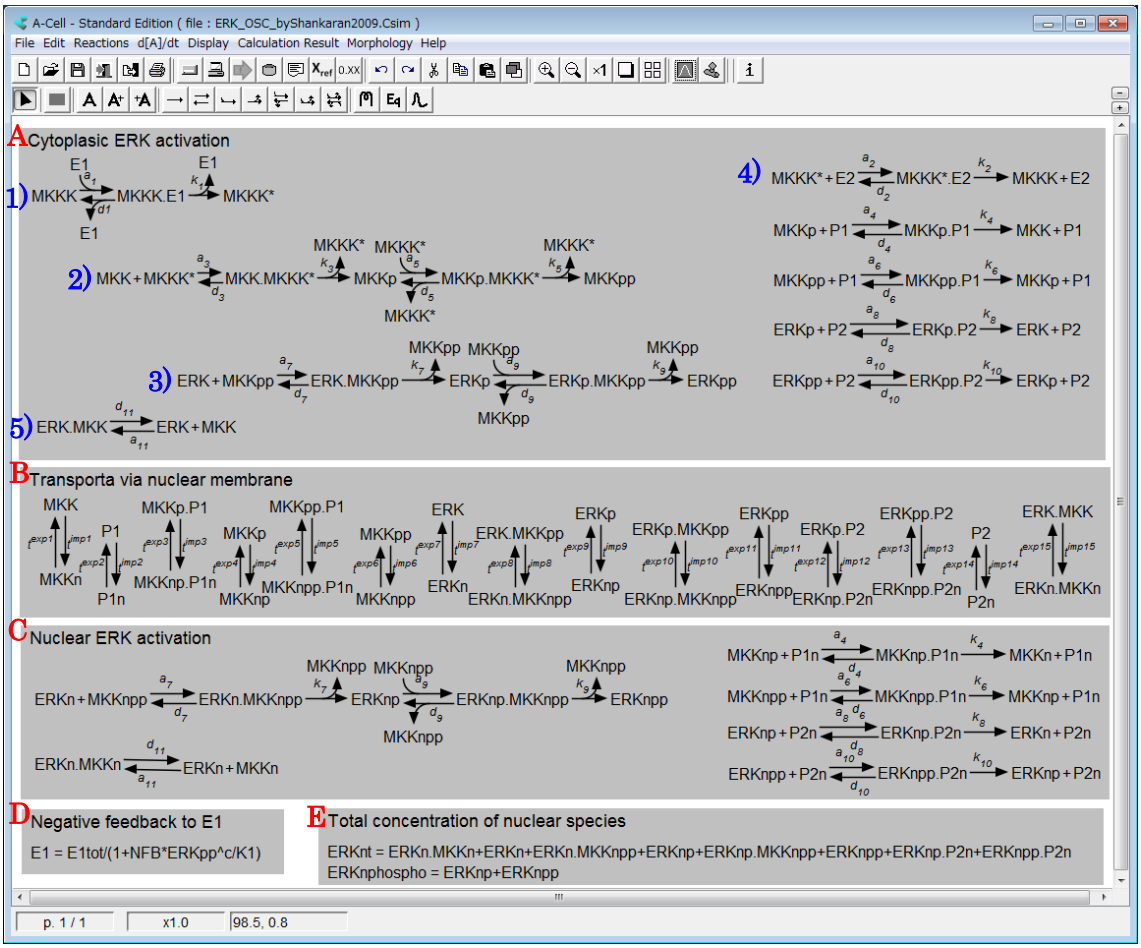

Figure 5 A-Cell reconstruction of a model by Shankaran et al[8].

This is the complete set of the Shankaran's model including negative feedback and nuclear transport. There are 5 groups, which are indicated by A to E in red characters: A is cytoplasmic reactions. B is nuclear transport. C is nuclear reactions. D is negative feedback. E is equations to calculate total concentrations.

In group A, 5 sets of reactions are show as indicated by 1) through 5) in blue characters. It is important to validate that these reactions are exact reconstruction of Figure 2 and 3. It is easily recognized that reaction 1) in Figure 5 is enzymatic reaction for MKKK activation by EGFR, which corresponds to reaction (1) in Figure 2. Reactions 2) in Figure 5 are enzymatic reactions for the

phosphorylation of MKK by active MKKK*. It is readily recognized that these are chain reactions, and thus the signaling structure is clearly shown, which was not easily recognized by a separated expression as in Figure 3. Reactions 3) in Figure 5 are also enzymatic reactions for the phosphorylation of ERK by active MKK (MKKpp). In reactions 4), there are 5 enzymatic reactions that correspond to reaction (2), (4), (6), (8), and (10) in Figure 2 describing dephosphorylation reactions. Finally, reaction 5) in Figure 5 corresponds to (11) in Figure 2 or 3. Thus all reactions in Figures 2 and 3 are reconstructed in the group A of Figure 5.

In group B, nucleocytoplasmic shuttling for 15 species is described in forms of biochemical reactions. Thus, the effect of N/C ratio, which was described using V in Eqs.2) and 3), is not described. Since this effect is modeled with much higher biological relevance by A-Cell 4D simulation, here we ignore this effect [3].

Group C describes nuclear reactions. All nuclear reactions in Figure 2 are reconstructed, which can be validated by comparing Figures 2 and 5.

Group D describes negative feedback model in a form of an equation as in Shankaran's model. Here $E1'_{tot}$ in Eq.4) is described by E1 for simplicity. "^" in the equation indicates power, and hence ERKpp^C equals to $ERKpp^C$. You can find an additional parameter NFB in the equation. Since there was a discussion on the mechanism of ERK oscillation about the requirement of negative feedback [6], [8], it is better to test this without changing the model. If you set NFB=1 or 0, E1 in the equation depends or does not depend on ERKpp, respectively. Thus the requirement of negative feedback on the ERK oscillation can be tested by setting values of NFB without changing the model. You can test this requirement by changing the value of NFB in the initial condition file of A-Cell (Cf. next Chapter).

Here, you may have a following question. E1 in reactions 1) of Group A is involved in the enzymatic reactions, and hence, E1 is a variable of differential equations. However, E1 in Group D is calculated by an equation, and hence, E1 value is independent from the differential equations in reaction 1) of Group A and solely fixed by the equation in Group D. This can be a serious inconsistency with respect to E1. It seems that the same problem was involved in the Shankaran's model [4]. This problem arises from the simplification of activation step of MKKK by EGFR. If biochemical reaction model including SOS, Ras, and other proteins in the course of MKKK activation from EGFR is constructed, SOS inactivation by active ERK are also described in biochemical reactions. This will give a consistent model. Within a range of the simplification of

[3] If you want to model the effect of N/C ratio as shown in Eqs2) and 3), you can describe Eqs.2) and 3) by using "equation group" of A-Cell. Or you can modify simulation program automatically generated by A-Cell, if you can write C language of computer programming. In spatio-temporal 4D simulation by A-Cell, these considerations are not required. This type of simulation will be published on our website.

[4] However, no description was found in Shankaran's paper.

signaling from EGFR to MKKK in the present model, however, calculation of E1 value by Eq.4) without any change by differential equations by reaction 1) in Group A might be a reasonable simplification. The reconstruction was carried out according to this assumption.

Group E describes equations calculating the total concentration of nuclear ERK and phosphorylated nuclear ERK. Since these total concentrations were used in Shankaran's simulation, the same total concentrations were calculated. Thus, we have confirmed that all models described in Figures 2 and 3 are reconstructed by A-Cell model shown in Figure 5.

5. ERK Pathway simulation by A-Cell

As described in Chapter 3, equilibrium concentrations for all molecular species should be obtained before running simulations. “Before” and “after” values in Table 1 indicate values before and after the equilibrium. As described in Chapter 3, “before” values were assigned to ERK, MKK, MKKK, P1, and P2 leaving others in zero. After a sufficiently long-time of simulation, where there was virtually no change in concentrations of all molecular species reaching equilibrium, we obtained “after” values, which were assigned as the initial concentration in the simulation of ERK signaling.

Table 1 All parameters and their values.

parameters	values		units	parameters	values		units
	before	after			before	after	
E1	0.00E+00	0.00E+00	M	c	1.00E+00	1.00E+00	
E1tot	2.50E-08	2.50E-08	M	NFB	1.00E+00	1.00E+00	
E2	1.20E-08	1.20E-08	M	d1	7.50E-02	7.50E-02	/s
ERK	4.80E-06	2.13E-06	M	d2	2.50E-02	2.50E-02	/s
ERK.MKK	0.00E+00	1.04E-06	M	d3	2.00E-01	2.00E-01	/s
ERK.MKKpp	0.00E+00	0.00E+00	M	d4	2.00E-01	2.00E-01	/s
ERKn	0.00E+00	1.50E-06	M	d5	2.00E-01	2.00E-01	/s
ERKn.MKKn	0.00E+00	1.34E-07	M	d6	2.00E-01	2.00E-01	/s
ERKn.MKKnpp	0.00E+00	0.00E+00	M	d7	2.00E-01	2.00E-01	/s
ERKnp	0.00E+00	0.00E+00	M	d8	2.00E-01	2.00E-01	/s
ERKnp.MKKnpp	0.00E+00	0.00E+00	M	d9	2.00E-01	2.00E-01	/s
ERKnp.P2n	0.00E+00	0.00E+00	M	d10	2.00E-01	2.00E-01	/s
ERKnphospho	0.00E+00	0.00E+00	M	d11	8.00E-01	8.00E-01	/s
ERKnpp	0.00E+00	0.00E+00	M	k1	1.75E-01	1.75E-01	/s
ERKnpp.P2n	0.00E+00	0.00E+00	M	k2	1.75E-01	1.75E-01	/s
ERKnt	0.00E+00	0.00E+00	M	k3	1.75E-01	1.75E-01	/s
ERKp	0.00E+00	0.00E+00	M	k4	1.75E-01	1.75E-01	/s
ERKp.MKKpp	0.00E+00	0.00E+00	M	k5	1.75E-01	1.75E-01	/s
ERKp.P2	0.00E+00	0.00E+00	M	k6	1.75E-01	1.75E-01	/s
ERKpp	0.00E+00	0.00E+00	M	k7	1.75E-01	1.75E-01	/s
ERKpp.P2	0.00E+00	0.00E+00	M	k8	1.75E-01	1.75E-01	/s
Ki	9.00E-09	1.00E-10	M	k9	1.75E-01	1.75E-01	/s
MKK	1.20E-06	2.34E-08	M	k10	1.75E-01	1.75E-01	/s
MKK.MKKK*	0.00E+00	0.00E+00	M	texp1	6.10E-01	6.10E-01	/s
MKKK	1.00E-07	1.00E-07	M	texp2	1.20E-02	1.20E-02	/s
MKKK*	0.00E+00	0.00E+00	M	texp3	2.60E-01	2.60E-01	/s
MKKK*.E2	0.00E+00	0.00E+00	M	texp4	5.40E-01	5.40E-01	/s
MKKK.E1	0.00E+00	0.00E+00	M	texp5	2.60E-01	2.60E-01	/s
MKKn	0.00E+00	4.22E-09	M	texp6	5.40E-01	5.40E-01	/s
MKKnp	0.00E+00	0.00E+00	M	texp7	1.80E-02	1.80E-02	/s
MKKnp.P1n	0.00E+00	0.00E+00	M	texp8	2.60E-01	2.60E-01	/s
MKKnpp	0.00E+00	0.00E+00	M	texp9	9.00E-03	9.00E-03	/s
MKKnpp.P1n	0.00E+00	0.00E+00	M	texp10	2.60E-01	2.60E-01	/s
MKKp	0.00E+00	0.00E+00	M	texp11	9.00E-03	9.00E-03	/s
MKKp.MKKK*	0.00E+00	0.00E+00	M	texp12	9.00E-03	9.00E-03	/s
MKKp.P1	0.00E+00	0.00E+00	M	texp13	9.00E-03	9.00E-03	/s
MKKpp	0.00E+00	0.00E+00	M	texp14	1.20E-02	1.20E-02	/s
MKKpp.P1	0.00E+00	0.00E+00	M	texp15	2.60E-01	2.60E-01	/s
P1	6.00E-08	3.00E-08	M	timp1	4.60E-02	4.60E-02	/s
P1n	0.00E+00	3.00E-08	M	timp2	1.20E-02	1.20E-02	/s
P2	5.00E-08	2.50E-08	M	timp3	3.50E-02	3.50E-02	/s
P2n	0.00E+00	2.50E-08	M	timp4	4.00E-02	4.00E-02	/s
a1	2.50E+07	2.50E+07	/M/s	timp5	3.50E-02	3.50E-02	/s
a2	2.50E+07	2.50E+07	/M/s	timp6	4.00E-02	4.00E-02	/s
a3	2.50E+07	2.50E+07	/M/s	timp7	1.20E-02	1.20E-02	/s
a4	2.50E+07	2.50E+07	/M/s	timp8	3.50E-02	3.50E-02	/s
a5	2.50E+07	2.50E+07	/M/s	timp9	1.20E-02	1.20E-02	/s
a6	2.50E+07	2.50E+07	/M/s	timp10	3.50E-02	3.50E-02	/s
a7	1.67E+07	1.67E+07	/M/s	timp11	1.20E-02	1.20E-02	/s
a8	2.50E+07	2.50E+07	/M/s	timp12	1.20E-02	1.20E-02	/s
a9	1.67E+07	1.67E+07	/M/s	timp13	1.20E-02	1.20E-02	/s
a10	2.50E+07	2.50E+07	/M/s	timp14	1.20E-02	1.20E-02	/s
a11	1.67E+07	1.67E+07	/M/s	timp15	3.50E-02	3.50E-02	/s

Green lines indicate symbols that were changed by a simulation acquiring the equilibrium. Shankaran et al. described that E1tot value was in a range from 2.5E-9 to 2.5E-7 M. In the reconstructed A-Cell model 2.5E-8 M was employed (gray line in Table 1). K_i value is shown in red line of Table 1. The value is different in "before" and "after" values. This is because ERK did not oscillate with the "before" value (9.0E-9 M), which was used in Shankaran's paper. Then the value was changed to "after" value (1.0E-10 M). The simulation result by A-Cell is shown in Figure 6.

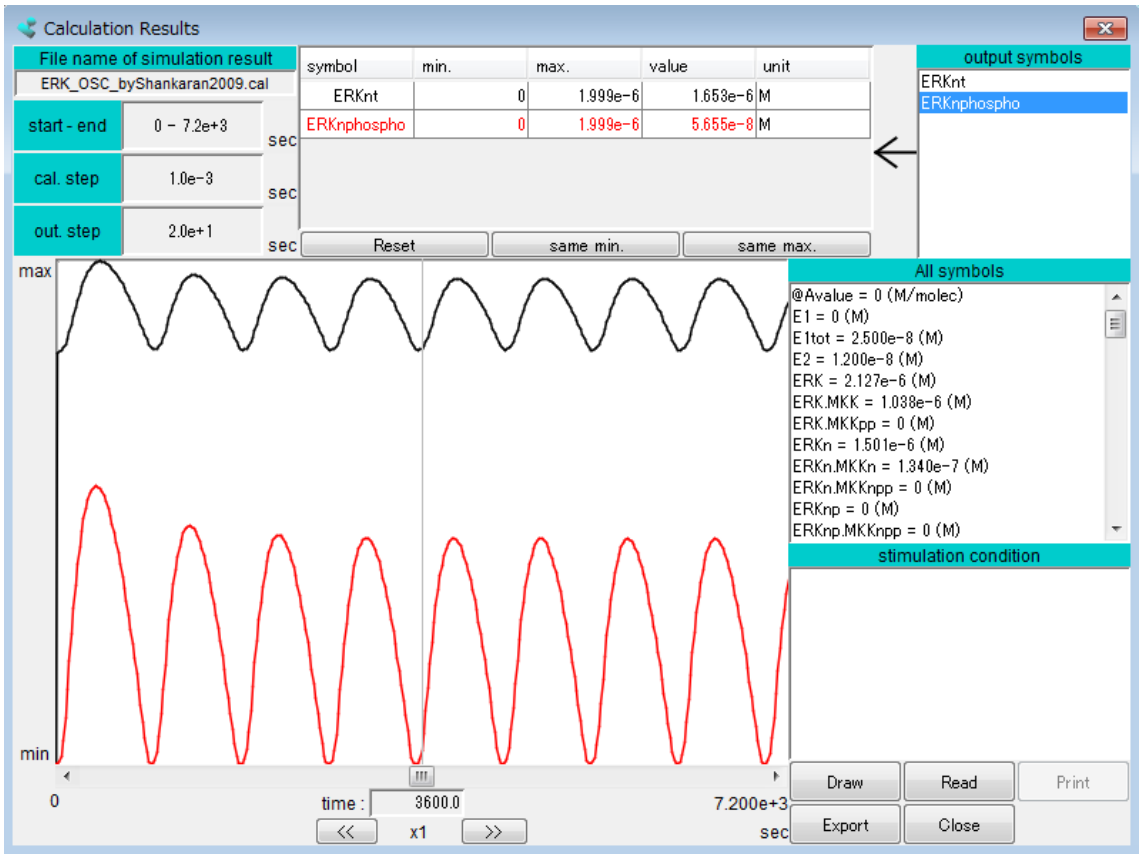

Figure 6 ERK pathway simulation by A-Cell.

This shows a result of an independent simulation, where calculation results by a simulation program generated by A-Cell was imported to A-Cell, and the time courses were drawn[5]. Phoshorylated nuclear ERK oscillates with a period of about 15 min as was shown by the Shankaran's paper (red line in Figure 6). The concentration of phosphorylated nuclear ERK was lower than that of total nuclear ERK as was reported by Shankaran et al. However, the peak nuclear value was larger than that shown in Shankaran's paper. This might be due to the change in the parameter values and/or neglecting N/C ratio in this A-Cell model, or others.

[5] Refer to "Introduction to A-Cell", which is downloadable from our website, for "independent simulation".

The simulation result shown in Figure 6 was not readily obtained because "before" value of K_i did not produce oscillation (Figure 7A). So we tried to find a reason and get new parameter values before acquiring simulation result in Figure 6.

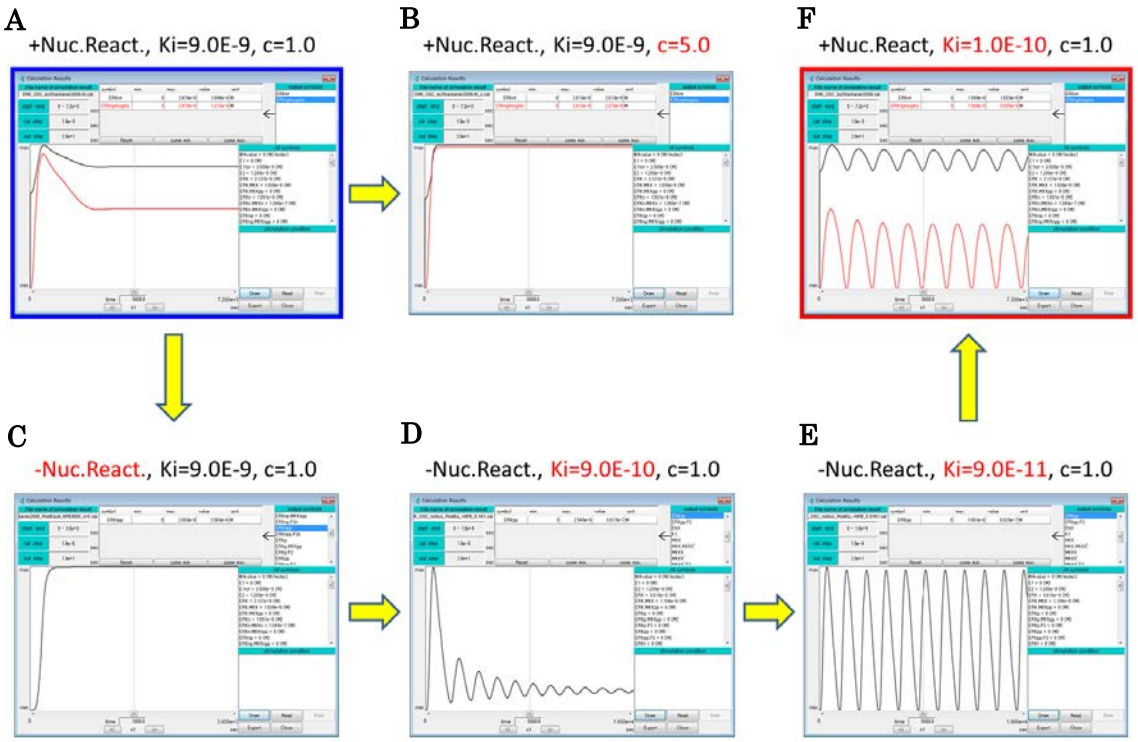

Figure 7 Process for obtaining ERK oscillation. Parameters shown in red indicate those that were changed from the previous settings.

In the course of finding parameters, hypothesis for the reason why we do not acquire desired result is important. Since there was a discussion on the role of negative feedback in ERK oscillation, first we hypothesized that something wrong in the parameter values in the equation of Group D of Figure 5. Then we changed c from 1.0 to 5.0. However, ERK did not oscillate (Figure 7B). If it is not easy to find parameter values, one approach is to simplify the model without affecting the essential behavior of the model. In the present case, we deleted nuclear reaction, because ERK oscillation was shown without nuclear reaction in the previous simulation report [4]. Again, no oscillation was observed by this simplification with the same parameters (Figure 7C). Next we reduced K_i value from 9.0E-9 to 9.0E-10 (Figure 7D). We found damped oscillations. Then we further reduced the K_i value to 9.0E-11 (Figure 7E). With this value, ERK oscillates persistently as was shown by Shankaran's simulation. Finally we define K_i value as 1.0E-10 for the model with nuclear reactions (Figure 7F), which is the same as Figure 6.

It is true that we made a detour to reach the goal (i.e. obtaining ERK oscillation). However, this

often occurs when reasoning for unexpected results was not easy. In such case, we should run trial-and-error simulations reaching to the desired result. Simplification is a powerful tool in the course of trial and error, and during this course, we find the direction, and finally reach to the goal. There are mathematical tools for efficient and/or semi-quantitative search parameters such as the sensitivity analysis. This might be discussed elsewhere.

6. How to Use Download File

A-Cell model shown in Figure 5 is downloadable from our website http://tc-simulations.com/homepage/a-cell/. You will find 5 files by unzipping a downloaded file (ERK_OSC.zip) as follows:

1) ERK_OSC_byShankaran2009.Csim
2) ERK_OSC_byShankaran2009.exe
3) ERK_OSC_byShankaran2009.cond
4) RK.TMP
5) ERK_OSC_byShankaran2009.cal

File 1) is the A-Cell model file, which can be imported to A-Cell Free Edition or higher. File 2) is the simulation program running on Windows PC. This is a compiled simulation program automatically generated by A-Cell. If you double click this file appeared on Windows Explorer, a simulation will start. The simulation creates the same file as 5). Files 3) and 4) should be in the same folder. These files contain initial condition for the simulation, and can be edited using a text editor such as Windows Notepad. By opening file 3) by a text editor, entries shown in Figure 8 appear. You can edit any entry. For example, 3rd line indicated by a green marker is the output file name, and different name can be specified. A value indicated by yellow marker is the initial value for E1tot. Different simulations can be run by changing the value. A symbol NFB can be found in this file. By changing its initial value from 1.0 to 0, simulation without negative feedback runs. Values indicated by light blue markers are time of calculation. These are (1) total calculation time, (2) calculation step, and (3) output step in second.

```
20
ERK_OSC_byShankaran2009.Csim
ERK_OSC_byShankaran2009.cal
107
@Avalue 0.0 M/molec C _
E1 0.0 M _ _
E1tot 2.5E-8 M _ _
E2 1.2E-8 M s _
ERK 2.12675E-6 M s _
ERK.MKK 1.03833E-6 M s _
        ⋮
t^i^m^p^9 0.012 /s C _
/*stimulation parameters*/
0
/*calculation condition*/
0.0
7200.0    ・・・・・(1)
0.001     ・・・・・(2)
20.0      ・・・・・(3)
/*number of output variables*/
2
        ⋮
```

Figure8 ERK_OSC_byShankaran2009.cond

File 5) is a simulation results file, which can be imported and displayed by A-Cell Entry Edition or higher. Simulation can be run by “in situ simulation” by all A-Cell Editions. However, it takes about 30 min with calculation step of 1e-2 sec for calculation time of 7,200 sec. However, there is 15% error in the total concentration of phosphorylated nuclear ERK. If calculation step is reduced to 1e-3 sec, a simulation requires 5 h with reduced error of 1.5%. If you use file 2), simulation time is expected to be reduced to 1/200.

Conclusions

As described in Introduction, we wrote this eBooklet for the purpose of reconstructing published simulation of ERK signaling by A-Cell, enabling modification of A-Cell model for further use by researchers who are engaged in ERK and related signaling. The reconstructed A-Cell model (Figure 5) replicated published simulation results with small differences. These differences might be due to the difference in some parameter values, modeling of nuclear transport, and/or other factors. The replication is the first step towards the 4D simulation by A-Cell, which is expected to give us novel findings.

The reconstruction of the published simulation is also an efficient process to capture the methods of creating novel pathway simulations. We will publish eBooklets on the reconstruction of published pathway simulations.

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