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Model analysis of difference between EGF pathway and FGF pathway

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Abstract

The difference in time course of Ras and mitogen activated protein kinase (MAPK) cascade by different growth factors is considered to be the cause of different cellular responses. We have developed the computer simulation of Ras-MAPK signal transduction pathway containing newly identified negative feedback system, Sprouty, and adaptor molecules. Unexpectedly, negative feedback system did not profoundly affect time course of MAPK activation. We propose the key role of fibroblast growth factor receptor substrate 2 (FRS2) in NGF/FGF pathway for sustained MAPK activation. More Grb2–SOS complexes were recruited to the plasma membrane by binding to membrane-bound FRS2 in FGF pathway than in EGF pathway and caused sustained activation of ERK. The EGF pathway with high concentration of EGF receptor also induced sustained MAPK activation, which is consistent with the results in the PC12 cell overexpressing the EGF receptors. The simulated time courses of FRS2 knock-out cells were consistent with those of the reported experimental results.

Keywords: Epidermal growth factor; Nerve growth factor; Fibroblast growth factor; Fibroblast growth factor receptor substrate 2

Signal transduction pathways are concerned with many cellular events including cell proliferation, differentiation, survival, and apoptosis. Even the same stimulation induces different cellular responses depending on cell types and stimulation time, although their signals are transduced through the same receptor and pathways. The growth factor receptors belonging to the family of protein–tyrosine kinase receptors also regulate cell growth, survival, and differentiation. These tyrosine kinases activate several signal transduction pathways, i.e., Janus kinase (JAK)/signal transducer and activator of transcription (STAT), phospholipase Cγ (PLCγ), phosphatidylinositol 3 kinase (PI3K), and Ras-mitogen activated protein kinase (MAPK) pathways.

The MAPK cascade initiated by Ras is a conserved pathway from yeast to human. It is activated by many growth factors and stimuli, and induced several responses. Activation of the MAPK cascade by receptor tyrosine kinases (RTKs), such as the epidermal growth factor (EGF) receptor, is initiated by binding of Shc and Grb2 to the phosphorylated tyrosine residues of the receptor. The complex of Grb2 and SOS activates Ras by GTP loading. Ras-GTP recruits Raf1 to the plasma membrane [1,2]. Then, Raf1 is phosphorylated and activated by not well-defined kinases with complex regulatory mechanisms [3–5]. Activated Raf then phosphorylates and activates the dual-specific kinase MEK, which phosphorylates and activates MAPK. This Ras-MAP kinase cascade is strictly regulated by several ways; for example, activation of Ras-GAP, phosphorylation of Raf, and inducible MAP kinase phosphatases (MKPs). Recently, we cloned a family of novel membrane-bound molecules, Spreds, which are related to Sprouty [6]. Spreds and Sproutys are shown to be negative regulators for several types of growth factor-induced MAPK activation, including the fibroblast growth factor (FGF) and EGF [7,8]. Four Sprouty homologs are found in mammals. Vertebrate Sproutys have also been implicated in the negative-feedback

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regulation of FGF-signaling in embryogenesis [9,10] and angiogenesis [11].

Ras-MAPK pathway is shown to be important for both cell proliferation and differentiation, and the strength or duration of MAP kinases is suggested to determine the cell fate. For example, EGF induces cell division in PC12 cells, but nerve growth factor (NGF) or FGF induces the differentiation of these cells. Although both EGF and NGF/FGF activate MAPK cascade, EGF causes transient activation of MAPK cascade, whilst NGF/FGF causes both transient and sustained activation. The dynamical difference is considered to be the cause of the difference in cell responses [12]. Cells with overexpressed EGF receptors showed transient and sustained activation of MAPK cascade in response to EGF and EGF induces differentiation of these transformants [13]. Although several computer models of Ras-MAPK pathway were developed [14–18], none of them clarified these differences.

In order to investigate the mechanism how different growth factors induce different time courses of MAP kinase activation, we have developed a computer simulation of the Ras-MAPK signal transduction pathways. The EGF and FGF pathways in PC12 cells were used. Our analysis indicates that fibroblast growth factor receptor substrate 2 (FRS2) shows the key role for the prolonged responses in the NGF/FGF pathway.

Model description

The kinetic scheme presented in Fig. 1(1) forms the basis for the computational analysis of the Ras-MAPK signaling network. This network is similar to that presented in previous models [14–18].

Briefly, the ligand binding induces the receptor dimerization and the autophosphorylation, which activates receptor's protein kinase activity. Phosphorylated tyrosine residues in the cytoplasmic domain of receptors work as binding sites for several adaptor proteins. Grb2 binds to a phosphorylated tyrosine residue of cytoplasmic domain of receptors and recruits GTP exchange protein for Ras, SOS. Activated Ras induces Raf phosphorylation. However, protein kinase for Raf phosphorylation has not been identified [3–5]. In this model, Ras is treated as virtual protein kinase for Raf. The phosphorylation of Raf induces MAPK cascade, MEK, and ERK phosphorylation. Activated ERK is translocated to the nucleus and phosphorylates several transcription factors, one example is Elk. Activated ERK also phosphorylates SOS and inactivates it. This inhibition makes one of the feedback loops of this pathway [19]. The ligand-induced endocytosis of receptors and the signal transduction by internalized receptors are included in EGF and FGF signal transduction pathway similar to the previous model [16–18].

In EGF pathway, Grb2–SOS complexes were recruited to the plasma membrane by binding to receptors directly or via Shc (Fig. 1(2)). The fibroblast growth factor receptor substrate 2 (FRS2) was reported to be myristylated and function as a lipid-anchored docking protein for Grb2–SOS complex without growth factor receptors in FGF signal transduction pathway [20,21]. FRS2 was reported to have SH2 binding site which was phosphorylated by activated FGF receptors [20] (Fig. 1(3)). The Grb2–SOS complex bound to phos-

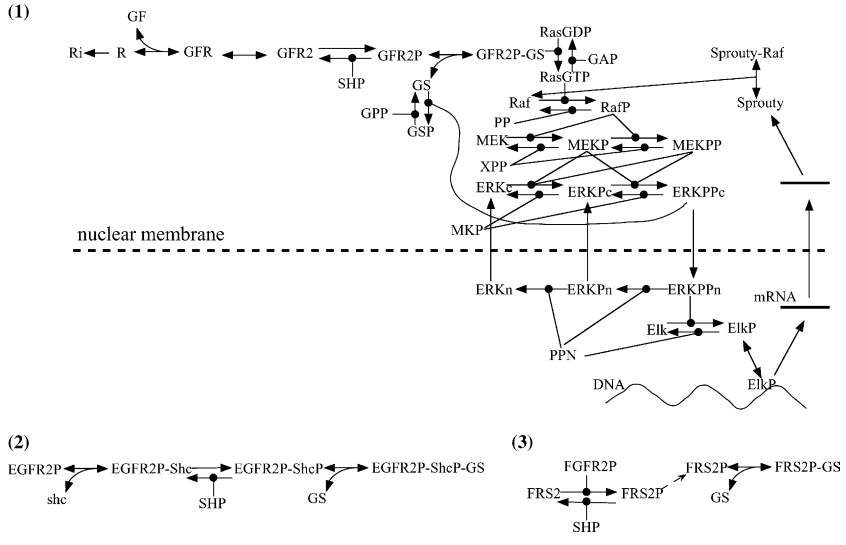

Fig. 1. Schematic representation of the computer simulation of Ras-MAPK signal transduction pathway. (1) The common pathway model between EGF and FGF, (2) signal transduction pathway from EGF receptor to Grb2–SOS, and (3) signal transduction pathway from FGF receptor to Grb2–SOS. In this model, arrows with dot symbols denote enzymatic reactions where enzymes are shown at the line to the dot symbols, and the name containing "P" usually denotes the phosphorylated type, e.g., "RafP" denotes the phosphorylated type of "Raf," the complex of some proteins is usually described in hyphen, e.g., the complex of "GFR2P" and "GS" is described in "GFR2P-GS."

phorylated FRS2, which is not bound to FGF receptor but directly bound to the plasma membrane via its myristyl group, also activates Ras-GDP. The concentration of FRS2 has not been precisely known, but we speculate that the amount of FRS2 is greater than FGF receptor, because FRS2 is a downstream amplifier of the FGF receptor signaling. The FRS2 was also reported to act as an adaptor protein in NGF signaling pathway [22].

Sprouty is induced by Ras-MAPK signal transduction pathway. We found Sprouty did not inhibit EGF pathway, but did inhibit FGF pathway [8]. Although its inhibitory mechanism has not been clarified, Sprouty did not inhibit the Ras activation, but inhibited the Raf activation [8]. In this model, Sprouty is included in the FGF pathway only and it binds to Raf to inhibit the Raf activation.

Parameter values and initial concentrations were set according to the values in the previous models [15,16]. But the models with such parameters did not show the time courses to fit observed time courses of EGF signal transduction pathway. Those values were set to fit the experimental data of EGF stimulation by using the genetic algorithm [23]. In FGF pathway, the same parameter values except those for FRS2 reactions were used.

In this kinetic analysis, Michaelis–Menten equation is not used, because in the signal transduction pathway the condition that the substrate concentration is much larger than the enzyme is not usually satisfied [24]. The transcription, translation, and translocation are approximated by the equations used in [24]. Detailed chemical reactions and their parameters are described in Appendix A. These reactions are described in the differential equations and solved mathematically by using Runge–Kutta–Gill method [24]. The simulation program was written in C by us, utilizing commonly used subroutine [24].

Results and discussion

The binding of growth factors induces receptor dimerization and the activation of their tyrosine kinase activity. The phosphorylated tyrosine residues work as the binding sites for several adaptor proteins having SH2 or SH3 domains. The Grb2 protein is one such adaptor protein. The SOS protein binds to plasma-membrane-bound Grb2 (bound to growth factor receptors in EGF pathway or to FRS2 in FGF pathway) and activates membrane-bound RasGDP by exchanging GDP with GTP. The activated Ras activates MAPK cascade (Raf-MEK-ERK1). The phosphorylated MAPK (ERK1) reached a maximum in 5–10 min and then decreased by the inhibitory phosphorylation of SOS by ERK1. In EGF pathway, phosphorylated ERK1 disappeared in 30–60 min. However, in FGF pathway, ERK activation was maintained for more than 2 h as shown in Fig. 2. Fig. 3 demonstrates that the simulated time course shows the above characteristics and the difference between EGF pathway (Fig. 3 left panel) and FGF pathway (Fig. 3 right panel). First, we thought that the induction of negative regulators may make the difference between MAPK activities in response to EGF and FGF. In FGF pathway, induced Sprouty inhibits Raf activation by binding to Ras–Raf complexes. Fig. 3 also shows the time course of Sprouty knock-out cells (dotted line), which were simulated by setting the mRNA synthesizing rate as 0, where the phosphorylation of ERK1 lasted for a longer time, activated Ras decreased faster than in the normal condition. This phenomenon can be explained by the Sprouty binding to Ras–Raf complexes. Ras–Raf–Sprouty complexes are considered to exist for a longer time [11]. However, the presence or absence of Sprouty could not explain the strong sustained activation of MAPK.

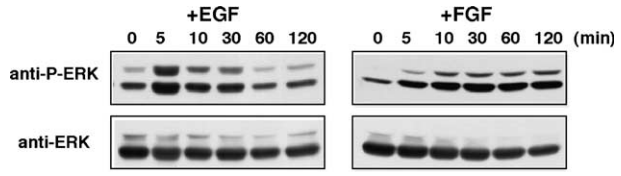

Fig. 2. Time course of MAPK (ERK) activation in response to EGF and FGF. Mouse embryonic fibroblasts (MEFs) were stimulated with 25 ng/ml EGF or bFGF for indicated periods. Cell extracts were prepared and analyzed with immunoblotting with anti-phosphoMAPK (ERK) (upper panel) and anti-ERK2 (lower panel).

It has been shown that phosphorylated FRS2 functions as an anchoring protein for Grb2 without binding to receptors. The difference between EGF pathway model and FGF one is the participation of FRS2 in FGF pathway, but not in EGF pathway. In order to clarify the FRS2's role, the dependency on FRS2 initial concentration was investigated. Figs. 4B and D shows the dependency on FRS2 concentration. In the case of lower FRS2 concentration, the time course of FGF pathway was similar to that of EGF pathway (compare Fig. 4B with Fig. 3K). Therefore, the prolonged phosphorylation of Elk or ERK1 was considered to be due to more Grb2–SOS complexes in plasma membrane which bound to the phosphorylated membrane-bound FRS2. On the other hand, the concentration of Shc in EGF pathway had no effect on the time course (Figs. 4A and C).

Although more sustained activities were observed without the receptor internalization, the difference between those with the internalization and those without was small (data not shown). The internalization could not explain the strong sustained activation of MAPK.

The time course of FRS2 knock-out cells showed no sustained activation (Fig. 5A) and low sensitivity to low concentration of FGF (Figs. 5A and B), which was consistent with the reported time course of FRS2

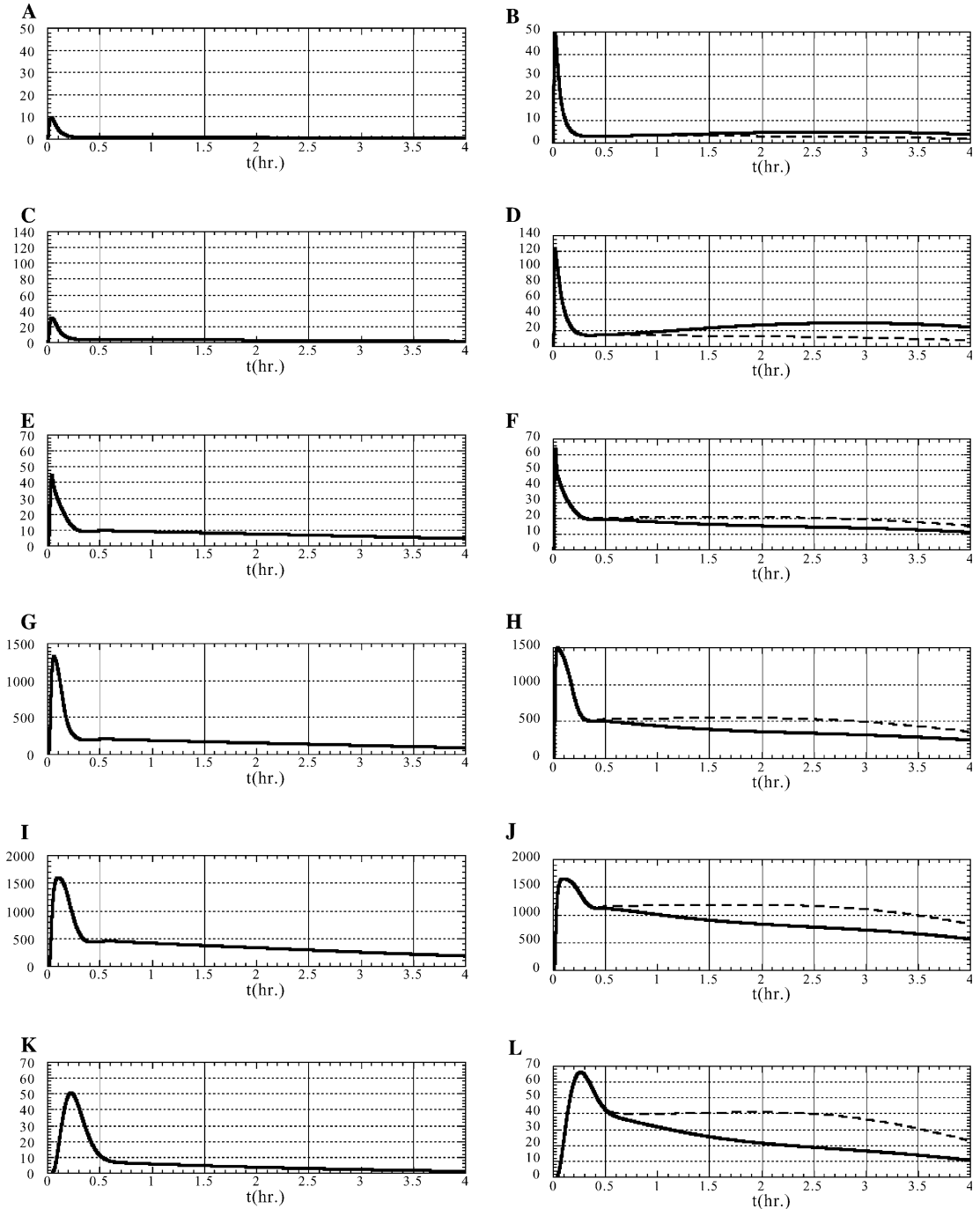

Fig. 3. Simulated time course of Ras-MAPK activation over 4 h continuous exposure to EGF (A, C, E, G, I, K) and FGF (B, D, F, H, J, L). The time course of GS (Grb2–SOS complex) bound to plasma membrane via EGFR (A) or phosphorylated FRS2 (B), activated Ras (RasT) (C,D), phosphorylated Raf (E,F), phosphorylated MEK (G,H), phosphorylated ERK (I,J), and phosphorylated Elk (K,L). The time courses of Sprouty knockout cells are also shown (dashed line in B, D, F, H, J, L).

knock-out cells [20]. In FGF pathway, since growth factor receptors act as a kinase for FRS2, FGF pathway shows hyper sensitivity to FGF concentration.

Another way to increase membrane-bound Grb2–SOS is to increase receptor concentration (normal concentration is 26 nM, the concentration is changed from 0.26 nM to 2.6 μM). Fig. 6 shows the dependency on the initial receptor concentration. As the initial concentration of EGF receptor increases, phosphorylation of MAPK is prolonged (Figs. 6A and C). This characteristic is consistent with the experimental responses of cells in which EGF receptor is overexpressed [12]. On the contrary, the increase in FGF receptor shows no increase in prolonged MAPK activation (Figs. 6B and D).

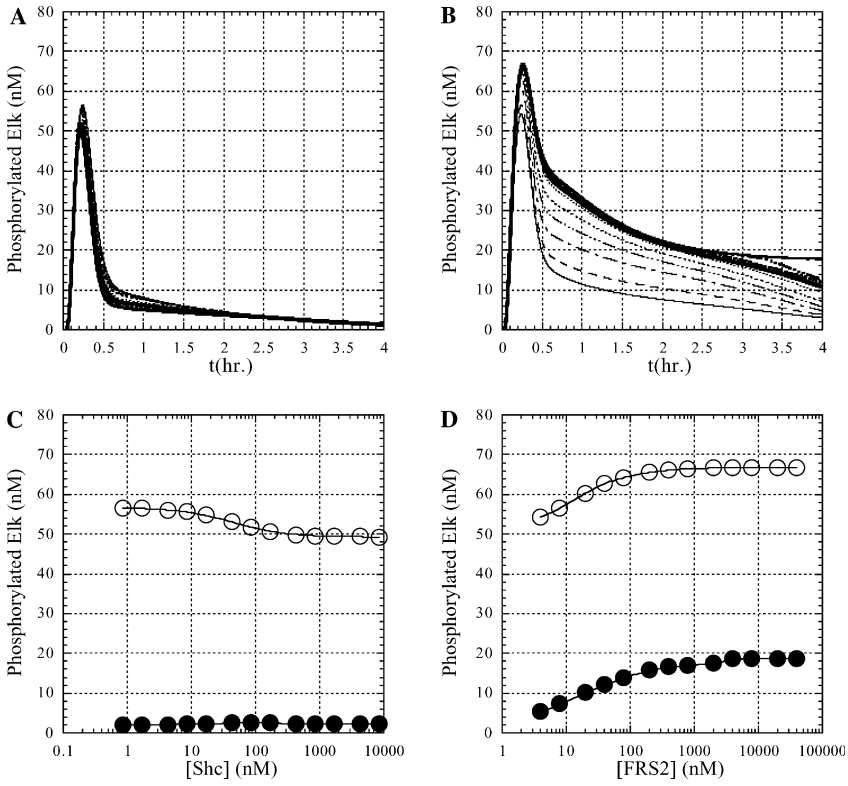

Fig. 4. Dependency on Shc and FRS2 concentration. (A,B) The time course of phosphorylated Elk for various concentrations (1/100–100 times) of Shc in EGF pathway (A) and FRS2 in FGF pathway (B). The bold line shows the time course of the normal condition. (C,D) The dependency of the peak concentration (○) and steady state (3 h) concentration (●) on Shc in EGF pathway (C) and on FRS2 in FGF pathway (D).

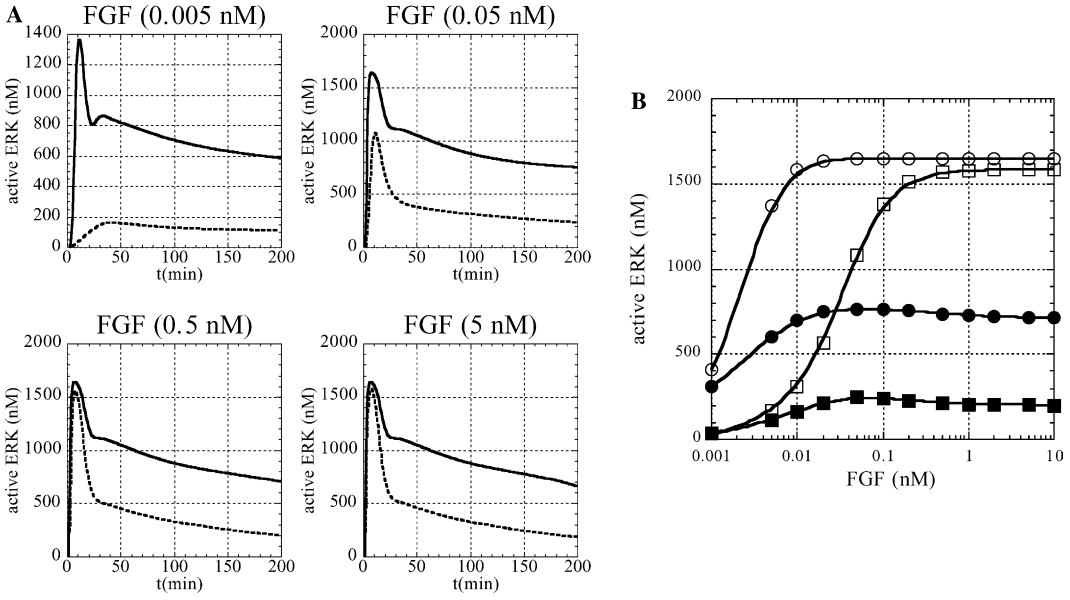

Fig. 5. Comparison of the time course of FRS2 knock-out cells with those normal cells. (A) The time course of phosphorylated ERK in the normal cells (solid line) and in the FRS2 knock-out cells (dashed line) stimulated by various FGF concentrations (0.005, 0.05, 0.5, and 5 nM). (B) The dependency on FGF concentration of peak values of phosphorylated ERK in the normal cells (○), that in the FRS2 knock-out cells (□), the steady state values (3 h) in the normal cells (●), and that in the FRS2 knock-out cells (■).

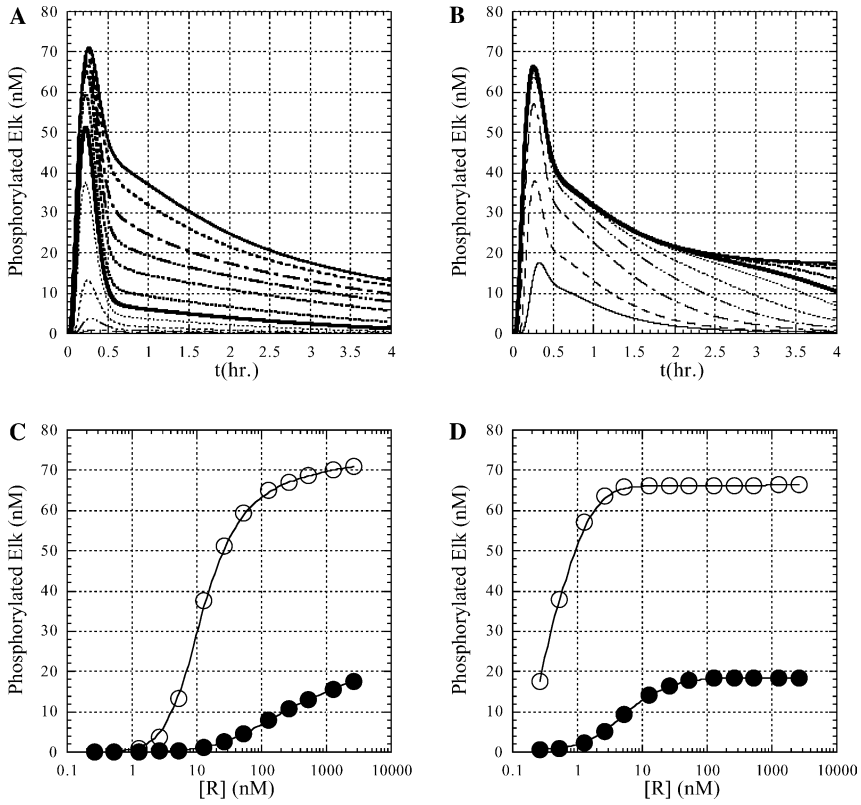

Fig. 6. Dependency on receptor concentration. The time course of phosphorylated Elk for various concentrations (1/100–100 times) of EGFR (A) and FGFR (B). The dependency of the peak concentration (○) and steady state (3 h) concentration (●) in EGF pathway (C) and FGF pathway (D).

Conclusions

Through a quantitative analysis of a computer simulation of Ras-MAPK signal transduction pathway, we have presented the key role of FRS2 in FGF pathway. In FGF pathway, more Grb2–SOS are recruited to the plasma membrane via phosphorylated FRS2 than those bound to receptors in EGF pathway. From our simulation study, the key difference between FGF pathway and EGF pathway is the concentration of Grb2–SOS complex bound to the plasma membrane. The increase in EGF receptor concentration is another way to increase membrane-bound Grb2–SOS. The model with the high EGF receptor concentration showed prolonged phosphorylation of ERK1 or Elk similar to FGF pathway, which was consistent with the result of the cell with overexpressed EGF receptors. FRS2 knock-out cells showed only transient activation, which was consistent with the experimental results [20]. Since the FRS2 was also reported to act as an adaptor protein in NGF signaling pathway [22], NGF signaling pathway is considered to have the same mechanism as FGF pathway.

Appendix A. Chemical reactions and their parameter values

First and second rate constants are expressed in units of s^{-1} and $10^6\,M^{-1}\,s^{-1}$, respectively. The dissociation constants (k_d) for binding reactions are also written in parentheses in units of nM. Initial concentrations of proteins are expressed in units of nM.

The following reactions are EGF pathway.

$[EGF] + [R] \leftrightarrow [EGFR]$ $k_1 = 100.$, $k_{-1} = 0.06$ ($k_{d1} = 0.6$)

$2[EGFR] \leftrightarrow [EGFR2]$ $k_2 = 10.$, $k_{-2} = 0.1$ ($k_{d2} = 10.$)

$[EGFR2] \rightarrow [EGFR2P]$ $k_3 = 2.014$

$[EGFR2P] + [SHP] \leftrightarrow [EGFR2P\text{-}SHP]$ $k_4 = 3.114$, $k_{-4} = 0.2$ ($k_{d4} = 64.22$)

$[EGFR2P\text{-}SHP] \rightarrow [EGFR2] + [SHP]$ $k_5 = 0.2661$

$[EGFR2P] + [Shc] \leftrightarrow [EGFR2P\text{-}Shc]$ $k_6 = 43.75$, $k_{-6} = 0.6$ ($k_{d6} = 13.72$)

$[EGFR2P\text{-}Shc] \rightarrow [EGFR2P\text{-}ShcP]$ $k_7 = 0.5838$

$[EGFR2P] + [ShcP] \leftrightarrow [EGFR2P\text{-}ShcP]$ $k_8 = 4.481$, $k_{-8} = 0.3$ ($k_{d8} = 66.95$)

[ShcP] + [SHP] ↔ [ShcP-SHP] k_4, k_{-4} (k_{d4})
[ShcP-SHP] → [Shc] + [SHP] k_5
[EGFR2P-ShcP] + [GS] ↔ [EGFR2P-ShcP-GS] $k_9 = 4.922$, $k_{-9} = 0.045$ ($k_{d9} = 9.143$)
[EGFR2P] + [GS] ↔ [EGFR2P-GS] $k_{10} = 2.734$, $k_{-10} = 0.025$ ($k_{d10} = 9.143$)
[RasD] + [EGFR2P-GS] ↔ [EGFR2P-GS-RasD] $k_{11} = 202.9$, $k_{-11} = 0.18$ ($k_{d11} = 0.887$)
[EGFR2P-GS-RasD] → [EGFR2P-GS] + [RasT] $k_{12} = 0.1434$
[RasD] + [EGFR2P-ShcP-GS] ↔ [EGFR2P-ShcP-GS-RasD] k_{11}, k_{-11} (k_{d11})
[EGFR2P-ShcP-GS-RasD] → [EGFR2P-GS]+[RasT] k_{12}
[RasT] + [GAP] ↔ [RasT-GAP] $k_{13} = 2.854$, $k_{-13} = 0.96$ ($k_{d13} = 336.4$)
[RasT-GAP] → [GAP] + [RasD] $k_{14} = 7.760$
[Raf] + [RasT] ↔ [Raf-RasT] $k_{15} = 1.754$, $k_{-15} = 0.05$ ($k_{d15} = 28.51$)
[Raf-RasT] → [RasT] + [RafP] $k_{16} = 0.07624$
[RafP] + [PP] ↔ [RafP-PP] $k_{17} = 4.908$, $k_{-17} = 0.4$ ($k_{d17} = 81.50$)
[RafP-PP] → [PP] + [Raf] $k_{18} = 0.3665$
[MEK] + [RafP] ↔ [MEK-RafP] $k_{19} = 9.196$, $k_{-19} = 0.9$ ($k_{d19} = 97.87$)
[MEK-RafP] → [MEKP] + [RafP] $k_{20} = 1.693$
[MEKP] + [RafP] ↔ [MEKP-RafP] k_{19}, k_{-19} (k_{d19})
[MEKP-RafP] → [MEKPP] + [RafP] k_{20}
[MEKPP] + [XPP] ↔ [MEKPP-XPP] $k_{21} = 2.060$, $k_{-21} = 0.4$ ($k_{d21} = 194.1$)
[MEKPP-XPP] → [MEKP] + [XPP] $k_{22} = 0.2752$
[MEKP] + [XPP] ↔ [MEKP-XPP] k_{21}, k_{-21} (k_{d21})
[MEKP-XPP] → [MEK] + [XPP] k_{22}
[ERKc] + [MEKPP] ↔ [ERKc-MEKPP] $k_{23} = 0.318$, $k_{-23} = 0.9$ ($k_{d23} = 2832.$)
[ERKc-MEKPP] → [ERKPc] + [MEKPP] $k_{24} = 0.1002$
[ERKPc] + [MEKPP] ↔ [ERKPc-MEKPP] k_{23}, k_{-23} (k_{d23})
[ERKPc-MEKPP] → [ERKPPc] + [MEKPP] k_{24}
[ERKPPc] + [MKP] ↔ [ERKPPc-MKP] $k_{25} = 0.239$, $k_{-25} = 0.4$ ($k_{d25} = 1676.$)
[ERKPPc-MKP] → [ERKPc] + [MKP] $k_{26} = 0.1298$
[ERKPc] + [MKP] ↔ [ERKPc-MKP] k_{25}, k_{-25} (k_{d25})
[ERKPc-MKP] → [ERKc] + [MKP] k_{26}
[GS] + [ERKPPc] ↔ [GS-ERKPPc] $k_{27} = 8.898$, $k_{-27} = 1.$ ($k_{d27} = 112.4$)
[GS-ERKPPc] → [GSP] + [ERKPPc] $k_{28} = 0.0426$
[GSP] + [GPP] ↔ [GSP-GPP] $k_{29} = 0.147$, $k_{-29} = 0.4$ ($k_{d29} = 2725.$)
[GSP-GPP] → [GS] + [GPP] $k_{30} = 0.01928$
[ERKPPc] → [ERKPPn] $k_{31} = 0.0002$
[Elk] + [ERKPPn] ↔ [Elk-ERKPPn] $k_{32} = 2.$, $k_{-32} = 1.$ ($k_{d32} = 500.$)
[Elk-ERKPPn] → [ElkP] + [ERKPPn] $k_{33} = 0.05$
[ElkP] + [PPN] ↔ [ElkP-PPN] $k_{34} = 1.$, $k_{-34} = 0.2$ ($k_{d34} = 200.$)
[ElkP-PPN] → [Elk] + [PPN] $k_{35} = 0.01$
[ERKPPn] + [PPN] ↔ [ERKPPn-PPN] k_{34}, k_{-34} (k_{d34})
[ERKPPn-PPN] → [ERKPn] + [PPN] k_{35}
[ERKPn] + [PPN] ↔ [ERKPn-PPN] k_{34}, k_{-34} (k_{d34})
[ERKPn-PPN] → [ERKn] + [PPN] k_{35}
[ERKPn] → [ERKPc] $k_{36} = 0.01$
[ERKn] → [ERKc] $k_{37} = 0.05$
[EGFR] → [EGFR]i $\mathrm{d}[\mathrm{EGFR}]\mathrm{i}/\mathrm{d}t = k_{38}*(e_1 + (1 - e_1) * (1 - \exp(-3t/T_1)))*[\mathrm{EGFR}]$ $k_{38} = 0.0024$, $e_1 = 0.12$, $T_1 = 195.$
[R]→[R]i $\mathrm{d}[R]\mathrm{i}/\mathrm{d}t = k_{39}*(e_1 + (1 - e_1)*(1 - \exp(-3t/\ T_1)))*[R]$ $k_{39} = 0.00012$, e_1, T_1
$\mathrm{d}/[R]\mathrm{i}/\mathrm{d}t = -k_{40}[R]\mathrm{i}$ $k_{40} = 0.005$

In FGF pathway, reactions concerning with Shc are excluded and reactions for FRS2 and for Sprouty are included. Other reactions except additional reactions below are same as reactions in EGF pathway. EGF in reactions should be exchanged into FGF for FGF pathway.

[FRS] + [FGFR2P] ↔ [FGFR2P-FRS] $k_{50} = 43.75$, $k_{-50} = 0.6$ ($k_{d50} = 13.72$)
[FGFR2P-FRS] → [FRSP] + [FGFR2P] $k_{51} = 2.584$
[FRSP] + [SHP] ↔ [FRSP-SHP] $k_{52} = 0.4$, $k_{-52} = 0.2$ ($k_{d52} = 500.$)
[FRSP-SHP] → [FRS] + [SHP] $k_{53} = 0.01$

$[\mathrm{FRSP}] + [\mathrm{GS}] \leftrightarrow [\mathrm{FRSP\text{-}GS}] \quad k_{54} = 10.94,\ k_{-54} = 0.1\ (k_{\mathrm{d}54} = 9.143)$
$[\mathrm{RasD}] + [\mathrm{FRSP\text{-}GS}] \leftrightarrow [\mathrm{FRSP\text{-}GS\text{-}RasD}] \quad k_{11},\ k_{-11}\ (k_{\mathrm{d}11})$
$[\mathrm{FRSP\text{-}GS\text{-}RasD}] \rightarrow [\mathrm{FRSP\text{-}GS}] + [\mathrm{RasT}]\ k_{12}$
$\mathrm{d}[\mathrm{mRNASn}]/\mathrm{d}t = k_{55a}[\mathrm{ElkP}]/(k_{55b} + [\mathrm{ElkP}]) \quad k_{55a} = 0.01\ \mathrm{nM/s},\ k_{55b} = 50\ \mathrm{nM}$
$[\mathrm{mRNASn}] \rightarrow [\mathrm{mRNASc}] \quad k_{56} = 0.001$
$\mathrm{d}[\mathrm{Sprouty}]/\mathrm{d}t = k_{57}[\mathrm{mRNASc}] \quad k_{57} = 0.02$
$\mathrm{d}[\mathrm{mRNASc}]/\mathrm{d}t = -k_{58}[\mathrm{mRNASc}] \quad k_{58} = 0.0005$
$\mathrm{d}[\mathrm{Sprouty}]/\mathrm{d}t = -k_{59}[\mathrm{Sprouty}] \quad k_{59} = 0.0001$
$[\mathrm{Sprouty}] + [\mathrm{Raf}] \leftrightarrow [\mathrm{Raf\text{-}Sprouty}] \quad k_{60} = 1.,\ k_{-60} = 0.1\ (k_{\mathrm{d}60} = 100.)$
$[\mathrm{Sprouty}] + [\mathrm{Raf\text{-}RasT}] \leftrightarrow [\mathrm{Raf\text{-}RasT\text{-}Sprouty}] \quad k_{60},\ k_{-60}\ (k_{\mathrm{d}60})$
$[\mathrm{Raf\text{-}Sprouty}] + [\mathrm{RasT}] \leftrightarrow [\mathrm{Raf\text{-}RasT\text{-}Sprouty}] \quad k_{15},\ k_{-15}\ (k_{\mathrm{d}15})$

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