STEADY STATE ANALYSIS OF SIGNAL RESPONSE IN RECEPTOR TRAFFICKING NETWORKS

ZHIKE ZI[1] EDDA KLIPP[1, 2]
zhike_zi@molgen.mpg.de klipp@molgen.mpg.de

[1]*Computational Systems Biology, Max Planck Institute for Molecular Genetics, Ihnestr. 73, 14195 Berlin, Germany*
[2]*Theoretical Biophysics, Humboldt University Berlin, Institute for Biology. Invalidenstr. 42, 10115 Berlin, Germany*

Receptor trafficking is used to describe the internalization and recycling processes of receptors in the cell. Considerable efforts of quantitative modeling have been made so far in the study of receptor trafficking networks. For the reason of simple mathematical analysis, the canonical receptor trafficking models either ignored the recycling step of receptors or didn't consider the trafficking of empty receptors. Here, we revisit the canonical receptor trafficking models and implement steady state analysis for a general model of receptor trafficking networks, which is composed of the *de novo* appearance of surface receptor, ligand-receptor interaction, internalization, recycling and degradation of both empty and occupied receptors. We present the analytical solution of the two steady states of the receptor trafficking networks before and after the network is exposed to the signal. The results indicate that the distribution of the empty receptor at the cell surface and inside of the cell, before signal is added, is mainly determined by the ratio of internalization rate and recycling rate of empty receptor. Furthermore, the steady state analysis demonstrates that classic Scatchard plot analysis is still valid for the steady state of the complicated receptor trafficking network.

Keywords: signal response; steady state analysis; receptor trafficking network.

1. Introduction

Cells communicate with their extracellular environment by the interaction between the receptors and ligand, which converts the information from the outside environment to inside cell responses such as cell proliferation, apoptosis, differentiation and growth. The receptors at cell surface can be internalized to early endosomes and also be recycled back, which is termed as receptor trafficking [1]. Receptor trafficking is implicated as a potential site for the regulation of signaling pathways by previous experimental data [2,3]. On the other hand, considerable efforts of quantitative modeling have been made in the study of receptor trafficking networks [4-8]. For the reason of simple mathematical analysis, the canonical receptor trafficking models either ignored the recycling step of receptors or didn't consider the trafficking of empty receptors [5,6]. In this work, we revisit the canonical receptor trafficking models and implement steady state analysis for the general model of receptor trafficking networks, which is composed of the *de novo* appearance of surface receptor, ligand-receptor interaction, internalization, recycling and degradation of both empty and occupied receptors. We show the analytical solution of two steady states of the receptor trafficking networks before and after the network is exposed to the signal and perform some qualitative analysis on the two steady states.

2. Mathematical Model of the Receptor Trafficking Network

Referring to the previous canonical models of receptor trafficking networks, we model all the processes with the law of mass action. The structure of the receptor trafficking network is illustrated in Fig. 1. We model the *de novo* synthesis of surface receptor as a constant rate of k_1. Extracellular ligand L can associate with free surface receptors Rs to form a receptor-ligand complex LRs with the forward rate constant k_2 and the ligand-receptor complex disassociates with the backward rate constant k_3. The empty and occupied surface receptors are internalized with the rate constants k_5 and k_7, respectively. Furthermore, the empty and occupied receptors inside the cell can be recycled back to cell surface with recycling rate constants k_4 and k_6, respectively. The degradation rate constants of empty and occupied receptors are set as k_9 and k_{10}, respectively. We also considered the possible dephosphorylation of the activated receptors with the rate constant k_8. The symbols of the parameters involved in the network are summarized in Table 1.

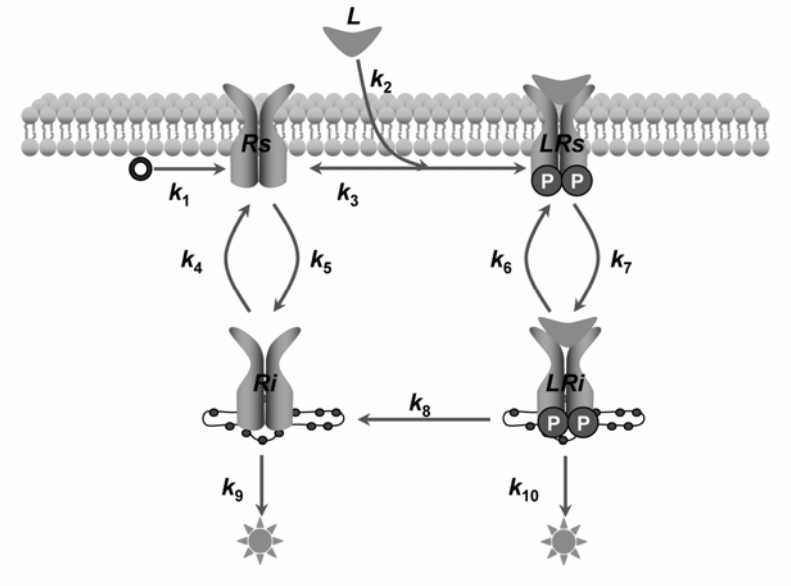

Fig. 1. Schematic description of the receptor trafficking networks. The network includes the de novo appearance of surface receptor, ligand-receptor interaction, internalization, recycling and degradation of both empty and occupied receptors. The symbols *L*, *Rs*, *LRs*, *Ri*, *LRi* represent ligand, cell surface empty receptor, cell surface ligand-receptor complex, internalized empty receptor and internalized ligand-receptor complex, respectively.

The ordinary differential equations for the components in the receptor trafficking networks can be written as

$$\frac{d[Rs]}{dt} = -(k_2[L] + k_5)[Rs] + k_4[Ri] + k_3[LRs] + k_1 \qquad (1)$$

$$\frac{d[Ri]}{dt} = k_5[Rs] - (k_4 + k_9)[Ri] + k_8[LRi] \quad (2)$$

$$\frac{d[LRs]}{dt} = k_2[L][Rs] - (k_3 + k_7)[LRs] + k_6[LRi] \quad (3)$$

$$\frac{d[LRi]}{dt} = k_7[LRs] - (k_6 + k_8 + k_{10})[LRi] \quad (4)$$

Table 1. Parameters for the model of the receptor trafficking network.

Symbols of Parameters	Corresponding Biological Processes	Typical Values	Reference
k_1	de novo synthesis of surface receptor	0.5 nM/min	[9]
k_2	formation of ligand-receptor complex	0.072 $nM^{-1}min^{-1}$	[8]
k_3	dissociation of ligand-receptor complex	0.34 min^{-1}	[8]
k_4	recycling of internalized empty receptor	0.2 min^{-1}	[10]
k_5	internalization of surface empty receptor	0.12 min^{-1}	[10]
k_6	recycling of internalized ligand-receptor complex	0.2 min^{-1}	[10]
k_7	internalization of surface ligand-receptor complex	0.15 min^{-1}	[10]
k_8	dephosphorylation of ligand-receptor complex	0.1 min^{-1}	
k_9	degradation of empty receptor	0.001 min^{-1}	[10]
k_{10}	degradation of ligand-receptor complex	0.01 min^{-1}	[10]

3. Steady State Analysis of the Receptor Trafficking Network

The concept of steady state is a mathematical idealization, which plays an important role in kinetic modeling [11]. A network is in steady state if the concentrations of the components do not change, which means the corresponding ordinary differential equations are zero. We can do a general steady state analysis of the receptor trafficking network by using only the network structure, without knowing the rate constants for a particular reaction [12].

3.1. Steady State of the Receptor Trafficking Network before the Signal is Added

If there is no ligand added to the receptor trafficking network, the concentration of ligand-receptor complexes *LRs* and *LRi* are zero. Therefore, the receptor trafficking network is composed of cell surface empty receptor and internalized empty receptor before the network is exposed to the external ligand. When the production, internalization, recycling and degradation of the empty receptors arrive at a steady state, we can derive the following system of algebraic equations for the empty receptors.

$$\frac{d[Rs]_1^{ss}}{dt} = -k_5[Rs]_1^{ss} + k_4[Ri]_1^{ss} + k_1 = 0 \quad (5)$$

$$\frac{d[Ri]_1^{ss}}{dt} = k_5[Rs]_1^{ss} - (k_4 + k_9)[Ri]_1^{ss} = 0 \quad (6)$$

The steady state concentrations of cell surface empty receptor *Rs* and internalized receptor *Ri*, obtained by solving the systems of algebraic equations (5-6), are

$$[Rs]_1^{ss} = \frac{k_1(k_4 + k_9)}{k_5 k_9} \tag{7}$$

$$[Ri]_1^{ss} = \frac{k_1}{k_9} \tag{8}$$

The steady state concentration of internalized empty receptor is determined by the synthesis rate and degradation rate of the receptor. The internalization and recycling steps of empty receptor affect the steady state concentration of surface receptor, but it has no effect on the steady state concentration of the empty receptor inside of the cell before the network is exposed to the ligand.

We are also interested in the distribution of the receptor at cell surface and inside of the cell. The distribution of the empty receptor can be evaluated by the ratio of internalized receptor to the surface receptor at steady state, which is

$$\frac{[Ri]_1^{ss}}{[Rs]_1^{ss}} = \frac{k_5}{k_4 + k_9} \approx \frac{k_5}{k_4} = \frac{internalization\ rate}{recycling\ rate} \tag{9}$$

Traditionally, the canonical receptor trafficking models usually ignored the trafficking of the empty receptor [4,7]. Such models assume that most of empty receptors exist at cell surface. From the expression of equation (9), we can see that the internalized receptors can be ignored only when the internalization rate of surface empty receptor is much smaller than the sum of the recycling and degradation rate of internalized empty receptor (ie. $k_5 << k_4+k_9$). The degradation rate of empty receptor is usually much smaller than the internalization rate ($k_9 << k_5$) [13]. Therefore, the distribution of empty receptors is mainly dependent on the ratio of internalization rate and recycling rate.

The assumption that most empty receptors distribute at cell surface will be valid when the recycling rate of empty receptor is much larger than the internalization rate. However, the internalization and recycling rate of receptor are various in different types of cells. For example, Burke *et. al* [10] experimentally measured the internalization rate of empty EGF receptor in HB2 and 184A1 cells, which is 0.03 min^{-1} and 0.15 min^{-1}, respectively. The corresponding recycling rate of empty receptor in HB2 and 184A1 cells is about 0.2 min^{-1} and 0.3 min^{-1}, respectively. From the result of steady state analysis, we can predict that the ratio of internalized empty EGF receptor to surface empty EGF receptor in HB2 cell is smaller than that in 184A1 cell. Therefore, the amount of internalized empty EGF receptors in 184A1 cells should not be ignored compared to the amount of the surface empty receptor. This prediction can be confirmed by the later

experimental data by Burke *et. al* [14]. For a quantitative study of receptor trafficking networks, it is necessary to check the validity of the assumption of ignoring the role of internalized empty receptors.

3.2. Steady State of the Receptor Trafficking Network after the Signal is Added

We next investigated the steady state of the receptor trafficking network after it is exposed to the external signal. When the ligand is added, the receptor trafficking network will reach another different steady state. According to the definition of steady state, we can derive the following system of algebraic equations for the new steady state:

$$\frac{d[Rs]_2^{ss}}{dt} = -(k_2[L]_2^{ss} + k_5)[Rs]_2^{ss} + k_4[Ri]_2^{ss} + k_3[LRs]_2^{ss} + k_1 = 0 \tag{10}$$

$$\frac{d[Ri]_2^{ss}}{dt} = k_5[Rs]_2^{ss} - (k_4 + k_9)[Ri]_2^{ss} + k_8[LRi]_2^{ss} = 0 \tag{11}$$

$$\frac{d[LRs]_2^{ss}}{dt} = k_2[L]_2^{ss}[Rs]_2^{ss} - (k_3 + k_7)[LRs]_2^{ss} + k_6[LRi]_2^{ss} = 0 \tag{12}$$

$$\frac{d[LRi]_2^{ss}}{dt} = k_7[LRs]_2^{ss} - (k_6 + k_8 + k_{10})[LRi]_2^{ss} = 0 \tag{13}$$

We can get the new steady state of the network by solving the system of algebraic equations (10-13), which leads to:

$$[Rs]_2^{ss} = \frac{k_1(k_4+k_9)(k_3(k_6+k_8+k_{10})+k_7(k_8+k_{10}))}{D} \tag{14}$$

$$[Ri]_2^{ss} = \frac{k_1(k_3k_5(k_6+k_8+k_{10})+k_2k_7k_8[L]_2^{ss}+k_5k_7(k_8+k_{10}))}{D} \tag{15}$$

$$[LRs]_2^{ss} = \frac{k_1k_2(k_4+k_9)(k_6+k_8+k_{10})[L]_2^{ss}}{D} \tag{16}$$

$$[LRi]_2^{ss} = \frac{k_1k_2k_7(k_4+k_9)[L]_2^{ss}}{D} \tag{17}$$

where

$$D = k_3k_5k_9(k_6 + k_8 + k_{10}) + k_7k_9(k_2[L]_2^{ss} + k_5)(k_8 + k_{10}) + k_2k_4k_7k_{10}[L]_2^{ss} \quad (18)$$

The expression of the new steady state of the receptor trafficking network is too complicated to be handled by mind. Here, we analyze the steady state concentrations of the components in the network under some special conditions and illustrate the effect of different parameters on the steady state of the network after it is exposed to the signal.

- Effect of Ligand: For the ligand concentration, it is usually assumed as a constant. Therefore, the steady state for the ligand (final concentration, $[L]_2^{ss}$) is a certain constant. On the other hand, if the amount of ligand is smaller than the amount of receptors and the ligand is degraded by the cell, the steady state of ligand ($[L]_2^{ss}$) will be zero. In this case, the activated ligand-receptor complex $[LRs]_2^{ss}$ and $[LRs]_2^{ss}$ will come to zero according to the equations (16-17). If we monitor the time course of the activated ligand-receptor complex, we will observe a transient signal response.
- Ratio of steady state concentration of internalized ligand-receptor complex to surface ligand-receptor complex: We can derive this ratio from equations (16-17) as

$$\frac{[LRi]_2^{ss}}{[LRs]_2^{ss}} = \frac{k_7}{k_6 + k_8 + k_{10}} \quad (19)$$

  This ratio is independent of ligand and the trafficking behavior of empty receptors.
- Effect of receptor synthesis rate (k_1): The receptor synthesis rate k_1 appears as a multiplier in the numerator, but not the denumerator, of the equations (14-17). Therefore, it is obviously that the faster the receptor synthesis rate, the higher value of the steady state of various forms of receptor in the receptor trafficking network.
- The total amount of occupied receptors is the sum of the internalized ligand-receptor complex and surface ligand-receptor complex, which is defined as bound receptors (Bound). From equations (16-17), we can derive

$$[Bound]_2^{ss} = [LRs]_2^{ss} + [LRi]_2^{ss} = \frac{\alpha[L]_2^{ss}}{\beta[L]_2^{ss} + \gamma} \quad (20)$$

  where

$$\alpha = k_1k_2(k_4 + k_9)(k_6 + k_7 + k_8 + k_{10}) \quad (21)$$

$$\beta = k_2k_7(k_4k_{10} + k_9(k_8 + k_{10})) \quad (22)$$

$$\gamma = k_5 k_9 (k_3 k_6 + (k_3 + k_7)(k_8 + k_{10})) \tag{23}$$

By this way, we can derive the Bound/Free and Bound relationship at steady state from equation (20):

$$\frac{[Bound]_2^{ss}}{[L]_2^{ss}} = -\frac{\beta}{\gamma}[Bound]_2^{ss} + \frac{\alpha}{\gamma} \tag{24}$$

The corresponding X-intercept for equation (24) is

$$\mathrm{X-intercept} = \frac{\alpha}{\beta} \tag{25}$$

Equation (24) is similar to the classical Scatchard plot (Rosenthal Plot). This result indicates that the classic Scatchard plot analysis is still valid for the steady state of complicated receptor trafficking network which includes ligand receptor interaction, internalization, recycling, dephosphorylation and degradation of receptors.

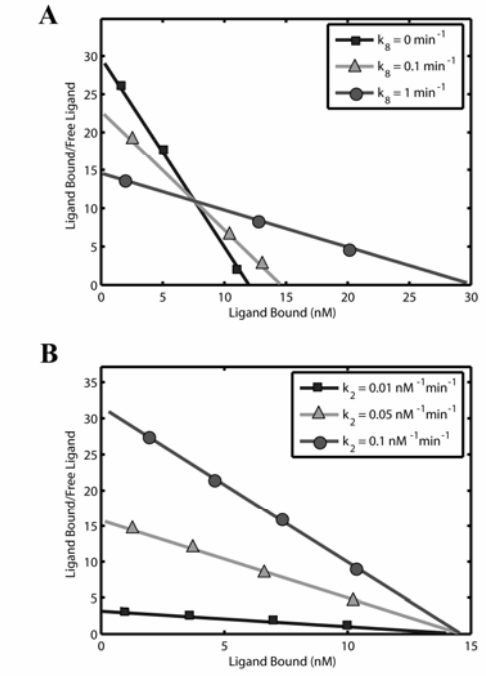

Fig. 2. Steady state Scatchard plot of the relationship between ligand bound and free ligand. The rate constants and corresponding typical values are list in Table 1. (A) Effect of different dephosphorylation rate of activated receptor complex (k_8). (B) Effect of different binding affinities (k_2).

- For the typical values of the parameters in the EGF receptor trafficking network (Table 1), the corresponding steady state binding plots with different dephosphorylation rates of activated receptor complex (k_8) are shown in Fig. 2A. The

slope of steady state Scatchard plot decreases with the increase of k_8. On the other hand, different binding affinities of ligand and receptor interaction are found in EGF receptor trafficking networks [15]. According to the equation (21-24), we can conclude that the higher the binding affinity of ligand and receptor interaction (ie. the higher value of k_2), the larger is the absolute value of slope in steady state Scatchard plot, which is confirmed by the computer simulation shown in Fig. 2B.

- When recycling steps of internalized receptors and deactivation of internalized receptor complex are ignored (k_4, k_6 and k_8 are zero) which is assumed in the early work of quantitative modeling of receptor trafficking network [4], we get the following expression

$$\frac{[Bound]_2^{ss}}{[L]_2^{ss}} = -\frac{k_2 k_7}{k_5(k_3 + k_7)}[Bound]_2^{ss} + \frac{k_1 k_2 (k_7 + k_{10})}{k_5 k_{10}(k_3 + k_7)} \tag{26}$$

Equation (26) is exactly the same expression as that derived by Wiley and Cunningham [4].

4. Conclusion

In this work, we implemented steady state analysis for the general receptor trafficking network before and after it is exposed to the external signal. We can draw the following conclusions based on the analysis results:

(1) Before ligand is added, the steady state concentration of internalized empty receptor is determined by the synthesis rate and degradation rate of the receptor. This is independent of the internalization and recycling rate of empty receptors.
(2) The distribution of the empty receptor at cell surface and inside of the cell, before signal is added, is mainly determined by the ratio of internalization rate and recycling rate of empty receptor.
(3) For quantitatively modeling of receptor trafficking networks, it is necessary to check the validation of the assumption of ignoring the role of internalized empty receptors because this assumption is only valid under a certain special condition.
(4) The classical Scatchard plot is still valid for the steady state of the complicated receptor trafficking network which includes ligand receptor interaction, internalization, recycling, dephosphorylation and degradation of receptors.

5. Acknowledgement

Z. Zi is supported by PhD program of the IMPRS for Computational Biology and Scientific Computing. This work was supported by a grant to E. Klipp., ENFIN, a Network of Excellence funded by the European Commission within its FP6 Programme, under the thematic area "Life sciences, genomics and biotechnology for health", contract number LSHG-CT-2005-518254.

References

[1] Moore, C.A., Milano, S.K., and Benovic, J.L., Regulation of receptor trafficking by GRKs and arrestins, *Annu. Rev. Physiol.*, 69451-69482, 2007.

[2] Schoeberl, B., Eichler-Jonsson, C., Gilles, E.D., and Muller, G., Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, *Nat. Biotechnol.*, 20(4): 370-375, 2002.

[3] Di Guglielmo, G.M., Le Roy, C., Goodfellow, A.F., and Wrana, J.L., Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover, *Nat. Cell Biol.*, 5(5): 410-421, 2003.

[4] Wiley, H.S. and Cunningham, D.D., A steady state model for analyzing the cellular binding, internalization and degradation of polypeptide ligands, *Cell*, 25(2): 433-440, 1981.

[5] Knauer, D.J., Wiley, H.S., and Cunningham, D.D., Relationship between epidermal growth factor receptor occupancy and mitogenic response. Quantitative analysis using a steady state model system, *J. Biol. Chem.*, 259(9): 5623-5631, 1984.

[6] Myers, A.C., Kovach, J.S., and Vuk-Pavlovic, S., Binding, internalization, and intracellular processing of protein ligands. Derivation of rate constants by computer modeling, *J. Biol. Chem.*, 262(14): 6494-6499, 1987.

[7] Lund, K.A., Opresko, L.K., Starbuck, C., Walsh, B.J., and Wiley, H.S., Quantitative analysis of the endocytic system involved in hormone-induced receptor internalization, *J. Biol. Chem.*, 265(26): 15713-15723, 1990.

[8] Starbuck, C. and Lauffenburger, D.A., Mathematical model for the effects of epidermal growth factor receptor trafficking dynamics on fibroblast proliferation responses, *Biotechnol. Prog.*, 8(2): 132-143, 1992.

[9] DeWitt, A.E., Dong, J.Y., Wiley, H.S., and Lauffenburger, D.A., Quantitative analysis of the EGF receptor autocrine system reveals cryptic regulation of cell response by ligand capture, *J. Cell Sci.*, 114(Pt 12): 2301-2313, 2001.

[10] Burke, P.M. and Wiley, H.S., Human mammary epithelial cells rapidly exchange empty EGFR between surface and intracellular pools, *J. Cell Physiol.*, 180(3): 448-460, 1999.

[11] Heinrich, R. and Schuster, S., *The Regulation of Cellular Systems*, Chapman and Hall, 1996.

[12] Kitano, H., Systems biology: a brief overview, *Science*, 295(5560): 1662-1664, 2002.

[13] Sorkin, A. and Waters, C.M., Endocytosis of growth factor receptors, *Bioessays*, 15(6): 375-382, 1993.

[14] Burke, P., Schooler, K., and Wiley, H.S., Regulation of epidermal growth factor receptor signaling by endocytosis and intracellular trafficking, *Mol. Biol. Cell*, 12(6): 1897-1910, 2001.

[15] Felder, S., LaVin, J., Ullrich, A., and Schlessinger, J., Kinetics of binding, endocytosis, and recycling of EGF receptor mutants, *J. Cell Biol.*, 117(1): 203-212, 1992.