Roles of regulated internalization in the polarization of cell surface receptors

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Abstract— **Cell polarization, the generation of cellular asymmetries, is a fundamental biological process. Polarity of different molecules can arise through several mechanisms. Among these, internalization has been shown to play an important role in the polarization of cell surface receptors. The internalization of cell surface receptors can be upregulated upon ligand binding. Additional regulatory mechanism can downregulate the internalization process. Here we describe a general model, which incorporates these two opposing processes, to study the role of internalization in the establishment of cell polarity. We find that the competition between these two processes is sufficient to induce receptor polarization. Our results show that regulated internalization provides additional regulation on polarization as well. In addition, we discuss applications of our model to the yeast system, which shows the capability and potential of the model.**

I. INTRODUCTION

Breaking cell symmetry is essential in diverse biological processes, including morphogenesis, asymmetric cell division, and cell migration. In response to internal or external cues, cells relocalize previously uniformly distributed molecular components to specific locations. For example, haploid cells of yeast form a new bud when grow vegetatively. They can also form a mating projection towards a cell of opposite mating type to initiate sexual reproductive cycles when grow with the presence of pheromone factor. In either case, yeast cells cease isotropic growth and go through a process of polarization, which leads to further morphological changes and complex functions.

There are several known mechanisms that can establish cell polarity. One mechanism is self-recruitment of relavent molecules. For example, experimental and computational results suggest that self-recruitment of the Cdc42 complex to the plasma membrane accounts for the spontaneous Cdc42 polarity in budding yeast [1] [2] [3] . Actin-polymerization dependent directed transport is another important mechanism, which was shown in several studies to polarize Cdc42 as well [4] [5] [6].

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It is not clear what role internalization (endocytosis), another fundamental biological process, plays in the establishment of cell polarity. However, studies have implicated that internalization is important for cell polarity in several ways. For example, it was shown that internalization can optimize the polarization of protein Cdc42 in budding yeast system by dynamically regulating the balance of internalization, diffusion and directed transport [7]. Internalization dependent recycling, which recycles the protein before polarity disperses, can maintain polarity of the protein when protein diffusion is slow [8]. Another study showed that endocytic corralling exocytic zone is required to stabilize the Cdc42 polarity [9].

Recently, internalization was found to play an important role in the establishment of pheromone receptor polarity in yeast cells [10]. The experiments showed that receptor internalization is regulated upon ligand binding through a complicated machinery. Mutations affecting internalization or regulation show dramatic defects in polarization and other biological functions. These experiments imply that internalization is essential in the polarization of yeast pheromone receptors. However, the mechanism of establishing cell polarity by internalization is not known. We describe here a general model on internalization and its regulation to study how regulated internalization can give rise to receptor polarity. To the best of our knowledge, our model is the first to study the role of internalization in cell polarity establishment, while existing computational models mainly focus on self-activation, recruitment, or directed transport of relevant molecules. We also applied the model to the yeast system. The results show that our model can account for the establishment of polarization of yeast pheromone receptors.

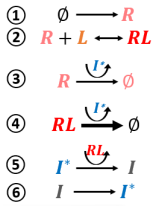
II. MODELS AND METHODS

A. Regulated receptor internalization

Cells polarize along the gradient direction of extracellular ligands. We assume ligands form a linear gradient, and we used a two-dimensional circle to model the cytoplasmic membrane of cells (Fig. 1). The cell membrane was discretized into segments. The ligand concentration in each segment was calculated based on the linear gradient assumption. In each segment, an identical reaction network was placed respecting to the local ligand input. Lateral diffusion among neighbor segments is considered in the model.

For simplicity, we considered only receptors and inhibitors that are involved in initiating the internalization of receptors, as well as their interactions in the reaction network. The polarization of receptors, both inactive and active, is used

as a indicator to measure the response of cells to the ligand gradient. The model is depicted in Fig. 2.



Receptors are synthesized and delivered onto membrane (Reaction 1). Without ligand binding (Reaction 2), receptors on the cell membrane are inactive and undergo constitutional internalization (basal internalization, Reaction 3). When receptors are bound by ligands, the internalization process is stimulated (Reaction 4), the rate of which was reported to be about 5- to 10-fold faster than basal internalization [11]. After internalization, inactive and active receptors will be destroyed through intracellular degradation (crossed dashed circle in Fig. 2). Both basal and stimulated internalization processes are initiated by the inhibitor of receptor (Inhibitor in Fig. 2). Active receptors can repress inhibitors through other pathways (Reaction 5).

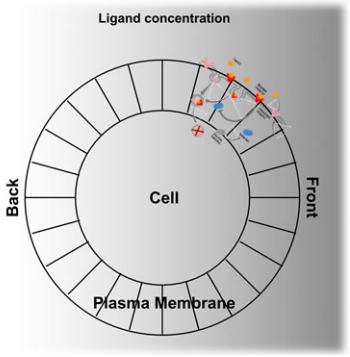


Fig. 1. 2D membrane model in gradient ligand environment. The darkness in the figure represents the concentration of ligand, where the ligand concentration is high on the gray side (front) and low on white side (back).

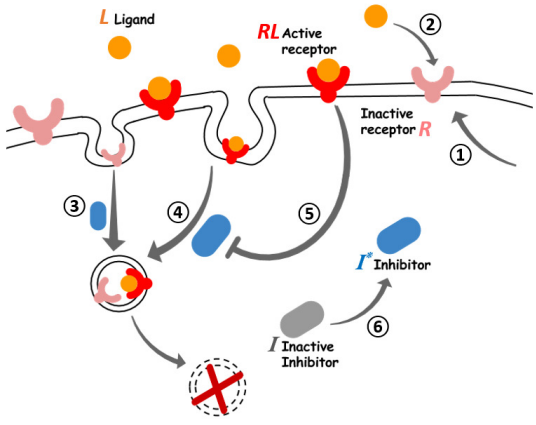


Fig. 2. The reaction network of regulated internalization model.

B. Mathematical model

The equations for our model are:

$$\frac{d[R]}{dt} = D\nabla^2[R] + k_{rs} + k_{rlm}[RL] - k_{rl}[R][L] - k_{i0}[I][R] \quad (1)$$

$$\frac{d[RL]}{dt} = D\nabla^2[RL] - k_{rlm}[RL] + k_{rl}[R][L] - \alpha \cdot k_{i0}[I][RL] \quad (2)$$

$$\frac{d[I]}{dt} = D\nabla^2[I] + k_{ia}([I_0] - [I]) - k_{ii}\frac{[RL]^n}{K^n + [RL]^n}[I][RL]. \quad (3)$$

Here $[R], [RL]$ and $[I]$ are the concentrations of inactive receptor, active receptor, and active inhibitor, respectively. These equations are written as partial derivative because we are to model how concentration of each molecular species changes in both time and space. In Eq. (1), the first term describes the diffusion of $[R]$, with D being the diffusion coefficient within the membrane. The second term, which is a constant, describes the synthesis rate of receptors. The third and forth terms represent the dissociation and association of receptors and ligands, respectively. The last term $-k_{i0}[I][RL]$ represents basal internalization of inactive receptors. Terms of Eq. (2) and (3) are similarly defined. For example, the term $-\alpha \cdot k_{i0}[I][RL]$ in Eq. (2) represents stimulated internalization of active receptors, where α is the stimulated ratio. The term $-k_{ii}\frac{[RL]^n}{K^n+[RL]^n}[I][RL]$ in Eq. (3) represents the repression of inhibitor, where k_{ii} represents inhibitor repression rate, and the Hill term $\frac{[RL]^n}{K^n+[RL]^n}[I][RL]$ simplifies cooperativity during repression of inhibitors from active receptors. n in the Hill term describes the cooperative strength.

The descriptions and values of parameters in the equations are summarized in Table I. Most of these values are taken or inferred from literatures. By varying α, k_{ii} and n separately, we show how polarity is established through regulated internalization and the roles that the corresponding interactions play. Simulation time is 50 min for all results, which resembles the experimental conditions.

III. RESULTS

A. Receptors polarize through stimulated internalization and repression of inhibitors

In our model, activation of receptors can trigger two opposing processes: upregulation of internalization from stimulated internalization and downregulation of internalization from repression of inhibitors. Our results show that the competition of these two opposing processes is essential for the establishment of the receptor polarity (Fig. 3).

Initially, both inhibitors and receptors are evenly distributed on the membrane. The concentration of receptors, both active and inactive, on both front and back sides begin to decrease after exposure to ligands due to the stimulated internalization (Fig. 3B). Since more active receptors are accumulated at the front of the cell, more inhibitors are

TABLE I

SUMMARY OF PARAMETERS

Param	Description	Value
D	Diffusion coefficient	$0.001\mu m^2/s$ [12]
k_{rs}	Synthesis rate of receptor	$0.08/\mu m^2 s$ [13]
k_{rlm}	Dissociation rate of receptor and ligand	$0.01/s$ [13]
k_{rl}	Association rate of receptor and ligand	$0.00332\mu m^3/s$ [13]
k_{i0}	Basal internalization rate	$5 \times 10^{-6}\mu m^2/s$ [13]
α	Internalization upregulation rate	10 [13]
k_{ia}	Activation rate of inactive inhibitor	$0.0001/s$
k_{ii}	Repression rate of inhibitor	$0.0005\mu m^2/s$
L	Ligand concentration	$5 - 10nM$
n	Cooperative strength in regulated internalization	10
K	Hill half-maximal constant	160
$[R_0]$	Initial concentration of inactive receptor	$200/\mu m^2$ $*$
$[RL_0]$	Initial concentration of active receptor	$0/\mu m^2$ †
$[I_0]$	Initial concentration of active inhibitor	$80/\mu m^2$

$*$ The value is estimated from [14]. † The value is estimated from [15]

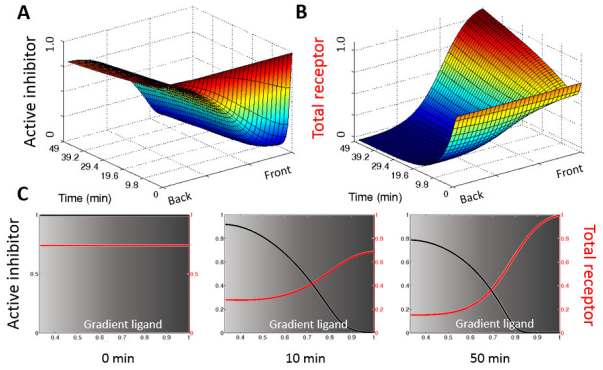


Fig. 3. Stimulated internalization and repression of inhibitors together can establish polarity. A. The spatio-temporal dynamics of the concentration of active inhibitors. x-axis is the cell polarity axis from the back to the front. y-axis shows time in minute. z-axis is the normalized concentration of molecular species. The concentration is color coded. B. The spatio-temporal dynamics of the concentration of total receptors. C. Concentration of active inhibitors and total receptors along the cell polarity axis at 0, 10 and 50min. The black curve represents active inhibitors, red total receptors. The background darkness shows the concentration of ligands. All results are normalized by the corresponding initial values.

repressed there, resulting in slower internalization. When internalization at the cell front is not fast enough to balance receptor synthesis, the receptor concentration increases again. On the back side of the cell, since the ligand concentration is low, there is no sufficient inhibitors get repressed, which leads to the eventual disappearance of receptors (Fig. 3).

B. Receptor polarity requires both stimulated internalization and repression of inhibitors

To test how the two opposing processes contribute to the receptor polarization, we turn off one process at a time to examine how cellular response are affected. This is achieved by setting α and k_{ii} to 0, alternatively. Without stimulated internalization ($\alpha = 0$), activation of receptors leads to only repression of inhibitors, as well as subsequent downregulation of the internalization. As a consequence, there are more receptors remain on the cell membrane at both front and back sides (Fig. 4A). On the other hand, when there is no repression of inhibitors ($k_{ii} = 0$), activation of receptors only leads to stimulated internalization, which leads to disappearance of receptors from the cell membrane at both front and back (Fig. 4B). These loss-of-function tests show that both stimulated internalization and repression of inhibitors are required to polarize receptors.

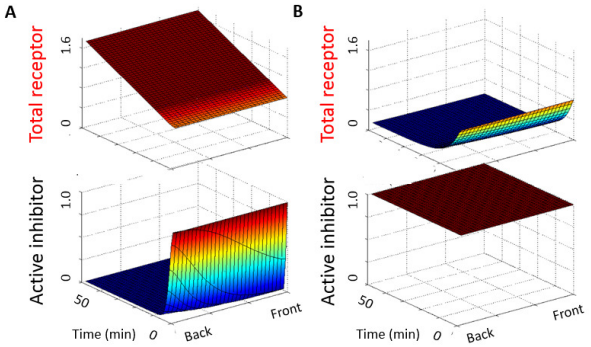


Fig. 4. Neither stimulated internalization nor repression of inhibitor alone can not form correct polarization.A. The spatio-temporal dynamics of total receptors and active inhibitors when there is no stimulated internalization. B. The spatio-temporal dynamics of total receptors and active inhibitors when there is repression of inhibitors.

C. Cooperativity provides an additional mechanism to regulate polarization

To quantify the extent of cell polarization, we define the polarization factor as: $pf = 1 - \frac{S_{1/2}}{S}$, where S is the total surface area of the cell, and $S_{1/2}$ is the surface area at the front of the cell that encompasses half of the total receptors (both inactive and active). If receptors are uniformly distributed on the cell surface, the polarization factor $pf = 0.5$. The more cells polarize, the closer to 1 is pf.

It has been shown that positive feedback plays an important role in breaking symmetry [4]. In the yeast mating system, it is suggested that certain positive feedback is also involved in the process of repression of internalization [10]. By varying cooperative strength n in the Hill term in our model (Eq. 3), we study how polarization is regulated by the cooperativity of the positive feedback. Results show that cell polarity increases with cooperative strength at the beginning, then decreases subsequently (Fig. 5 upper panel). When n is small, the cooperativity is not strong, and the repression of inhibitor is not sufficient even at the cell front to have cell polarization. Receptors at both front and back disappear from cell surface. When the cooperative strength is sufficiently large, cells polarize according to the extracellular clue. When cooperative strength is exceedingly large, even inhibitors at back are repressed and the concentration of receptors increases everywhere (Fig. 5 lower panel). The cooperativity in the repression of inhibitors therefore provides an additional mechanism to regulate the degree of polarization.

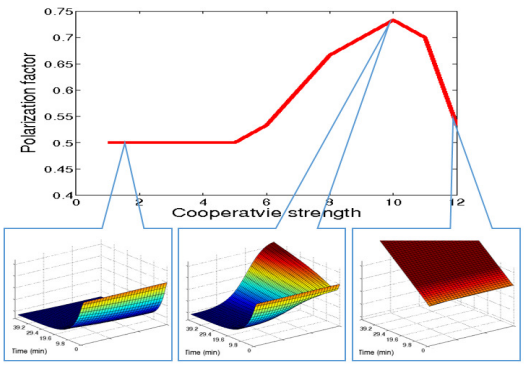


Fig. 5. Cooperativity affects cell polarity. Upper panel. How cell polarity changes with cooperative strength n. Lower panel. The typical spatio-temporal response of cell surface receptors for different cooperative strength.

D. A new model of polarization of yeast pheromone receptor induced by mating pheromone gradients

We developed a general model to study the roles of internalization in cell polarity. Our model is motivated by studies in yeast system. This general model can be useful to study the problem of yeast pheromone receptor polarization [10]. The connection between the general model and specific yeast system can be seen from the reaction networks (Fig. 6).

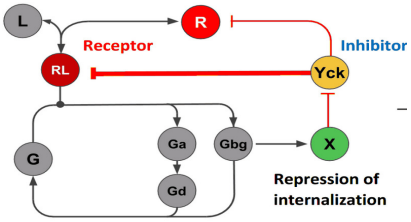


Fig. 6. The reaction network of yeast pheromone receptor system. Yck initiates the internalization of inactive (R) and active receptors (RL). Activation of receptors triggers the activation of the G protein cycle, then represses the inhibitor Yck through a factor X [10], thus represses internalization.

In the yeast model, the activation of receptors triggers the activation of the G protein cycle, which in turn repress kinase Yck which plays the role of inhibitor. The results show the establishment of polarity of yeast pheromone receptors (Fig. 7). In addition, the time scale agrees well with the experimental data [10].

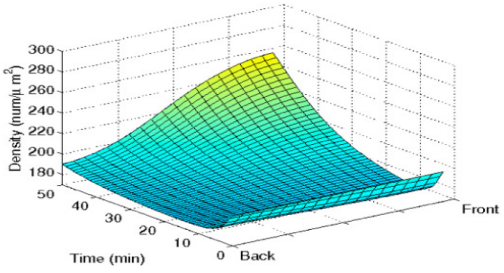


Fig. 7. Spatio-temporal response of yeast pheromone receptors. At 50 min, it shows clear polarity which agrees with experimental results.

IV. CONCLUSIONS

Here we present a general model incorporating stimulated internalization of cell membrane receptors and repression of the internalization, which is inspired by recent experimental studies in the yeast system. Our model revealed a novel mechanism that may be important for the polarization of receptors. We found that when cells are exposed to ligand gradient, the competition between stimulated internalization and repression of internalization is sufficient to induce receptor polarization. Furthermore, our results show that cooperativity in regulated internalization provides an additional mechanism in regulating cell polarization.

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