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LA PHYSIQUE À L'ÉCHELLE DE LA CELLULE
THE PHYSICS AT THE SCALE OF THE CELL

Robust amplification in adaptive signal transduction networks

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Abstract. Amplification of small changes in input signals is an essential feature of many biological signal transduction systems. An important problem is how sensitivity amplification can be reconciled with wide dynamic range of response. Here a general molecular mechanism is proposed, in which both high amplification and wide dynamic range of a sensory system is obtained, and this without fine-tuning of biochemical parameters. The amplification mechanism is based on inhibition of the enzymatic activity of the sensory complex. As an example, we show how this 'inhibition-driven amplification' mechanism might function in the bacterial chemotaxis network, where it could explain several intriguing experimental observations connected with the existence of high gain, wide dynamic range and robust adaptation. © 2001 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

transduction / amplification / robustness / networks

Robuste amplification dans les réseaux adaptifs de transduction du signal

Résumé. *Une des caractéristiques principales de la transduction du signal en biologie est la très grande amplification de faibles changements des signaux d'entrée. Cette amplification doit être compatible avec de très grandes plages dynamiques de fonctionnement. Nous proposons ici un mécanisme général dans lequel une forte amplification et une très grande plage dynamique du système sensoriel sont obtenues. Ce mécanisme ne nécéssite pas d'ajustement précis des paramètres biochimiques. Il est fondé sur l'inhibition de l'activité enzymatique du complexe sensoriel. En exemple, nous montrons comment cette « amplification par inhibition », pourrait être à la base du fonctionnement du réseau chémotactique des bactéries. Il pourrait expliquer plusieurs observations expérimentales surprenantes, liées à l'existence d'un gain fort, d'une grande plage dynamique et d'une adaptation robuste. © 2001 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS*

transduction / amplification / robustesse / réseaux

1. Introduction

Cells sense and respond to changes in their environment using networks of interacting enzymes. An important feature of these signal transduction networks is their ability to amplify small incoming signals.

Note présentée par Guy LAVAL.

The magnitude of a stimulus can readily be increased by many catalytic processes. In contrast, 'sensitivity amplification' in which relative changes in the stimulus are amplified, requires more sophisticated molecular mechanisms [1]. Several such mechanisms were proposed [2], based on cooperativity in single proteins, receptor clustering [3], multiple inputs in enzymatic cascades [4], zero-order ultra-sensitivity [5] or branch-point amplification [6].

The larger the sensitivity amplification, the more switch-like is the response of the system (*figure 1*). High amplification thus comes at a price: changes in the input signal are detected only in a narrow range around the threshold of the switch [1]. In order to detect changes over a wide range of background levels (wide 'dynamic range'), sensory systems rely on adaptation. The adaptation processes bring the system back to the vicinity of the amplifier threshold after each stimulus [1]. Such a combination between adaptation and amplification processes, however, would appear to depend on delicate adjustment of biochemical parameters, to ensure that the adapted steady state lies very close to the amplifier threshold. This raises a general problem of robustness: The ability to amplify would be lost upon small variations in the enzyme rate constants and concentrations. It is thus difficult to imagine how one could reconcile high amplification, wide dynamic range and robustness [7] within a sensory system.

Here we propose a new mechanism for sensitivity amplification in biochemical signaling networks. It is based on the inhibition of the enzymatic activity of sensory complexes. Within this 'inhibition-driven amplification' scheme, an alteration of any of the biochemical parameters will cause a coordinated change in both the amplifier threshold and the adapted steady state of the network, keeping the system in the range of high amplification. As a result, the network displays both high gain and wide dynamic range without precise adjustment of its biochemical parameters.

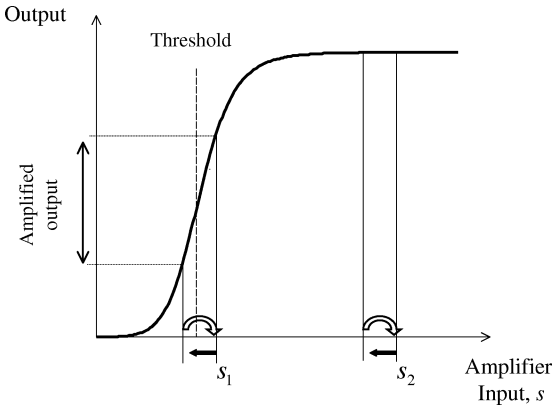

Figure 1. A typical input–output curve for an amplifying system. When the steady state input (s_1) is close to the threshold, a small stimulus (black arrow) leads to a large response. Adaptation (open arrow) resets the input back to the vicinity of the threshold and maintains the ability to respond to further stimuli. When the steady state is away from the threshold (s_2) the ability to respond to small signals is severely reduced. Amplification generally relies on 'fine tuning': the biochemical parameters must be carefully adjusted so that the steady state maintained by the adaptation process lies at the threshold of the amplifier.

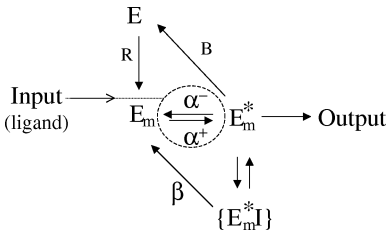

Figure 2. Two-state model exhibiting robust adaptation and amplification. A receptor E is modified by enzyme R, which works at saturation. The modified receptor undergoes transitions between active and inactive states, denoted by E_m^* and E_m respectively, with transition rates $\alpha^{\pm}$ that depends on the input level ℓ. E_m^*, but not E_m, is the substrate for enzyme B which catalyses the reverse modification reaction. An inhibitor I binds strongly to the active receptor E_m^*. The complex $\{E_m^* I\}$ cannot transmit an output signal. It dissociates with a rate β, releasing an inactive receptor. There are three time scales in the system. Modification reactions are slow, transition between active and inactive receptors are intermediate, while the binding of inhibitor is fast.

We begin by describing inhibition-driven amplification in a simple sensory system (*figure 2*). The receptor in this system has two functional states: active and inactive. The sensory input, binding of a ligand to the receptor, affects the transition rates between these states and thus shifts the balance between them. In the active state the receptor can potentially transmit a signal to a downstream messenger, for instance by phosphorylation. However, this can be prevented by an inhibitor molecule that binds strongly and exclusively to the active receptor. The output of the network is thus the concentration of active receptors that are not in complex with the inhibitor, and are free to interact with downstream messengers.

A slow adaptation process, following the response to a change in ligand, resets the signaling output. Adaptation is due to a reversible modification of the receptor, for example through methylation or phosphorylation. This modification can compensate for the effects of ligand binding. We assume that the enzyme, which removes the modification, acts only on the uninhibited active receptors. It can be then shown that after stimulation the output always returns to exactly the same steady state, a feature called 'exact adaptation' [7]. The mechanism requires that the system works far from equilibrium. The necessary free energy input could, for example, be provided by a coupling of the transition $E_m \rightarrow E_m^*$ to ATP hydrolysis.

The amplification of the system can be evaluated by increasing the ligand concentration, ℓ, by $\Delta\ell$, and measuring the resulting change in the output signal ΔA from its steady state value, $A^{\rm st}$. We define the gain, g, as the ratio between the fractional change in the output signal and the fractional change in the number of unoccupied receptors:

$$g \equiv \frac{\Delta A/A^{\rm st}}{\Delta\ell/(1+\ell)} \tag{1}$$

where ℓ is measured in units of the receptor dissociation constant. The maximal gain of isolated receptors, without the rest of the network, would be $g = 1$, so that, for instance, a 1% change in unoccupied receptors would result in a 1% change in output. Similarly, the maximal gain of the network without the inhibitor is also $g = 1$. The full system, however, shows a much larger sensitivity amplification. It can be shown (see Section 2) that the shift of the balance between the active and inactive receptor states, induced by addition of ligand, comes mainly at the expense of the small fraction of active receptors which are uninhibited. Thus, a small addition of ligand can lead to a large change in signaling output. For instance, consider the case when the total number of active receptors is ten fold larger than the number of uninhibited ones. Although a 1% change in the number of receptors that bind ligand corresponds to a 1% change in the total number of active receptors, it may actually result in a 10% change in the number of *uninhibited* active receptors and hence the output signal. More precisely, if the inhibitor concentration, $I_{\rm tot}$ is much larger than the steady state concentration of uninhibited active receptors, $A^{\rm st}$, then the maximal gain is given by:

$$g = r\frac{I_{\rm tot}}{A^{\rm st}} \gg 1 \tag{2}$$

where $r = \beta/\alpha^-$ is the ratio between two rate constants defined in *figure 2*.

The requirement for high amplification, $I_{\rm tot} \gg A^{\rm st}$, means that the total number of active receptors is only slightly higher than the number of inhibitor molecules. Naively, this would seem to imply a delicate adjustment of biochemical parameters. However, such fine-tuning is not needed. First, the adaptation mechanism ensures that at steady state there is always an excess of uninhibited active receptors. Second, the conditions for this excess to be small and thus for high gain, depend only on having large ratios between certain parameters and not on their delicate adjustment. High gain is thus maintained for a wide range of enzyme concentrations and kinetic rate constants.

As an example, we show how the proposed inhibition-driven amplification mechanism may apply to bacterial chemotaxis. Bacteria such as *E. coli* are highly efficient in detecting and swimming up gradients of attractant molecules, even when these are imposed on large backgrounds [8,9]. The bacteria display chemotaxis over five orders of magnitude of attractant concentrations [10] and show a measurable response

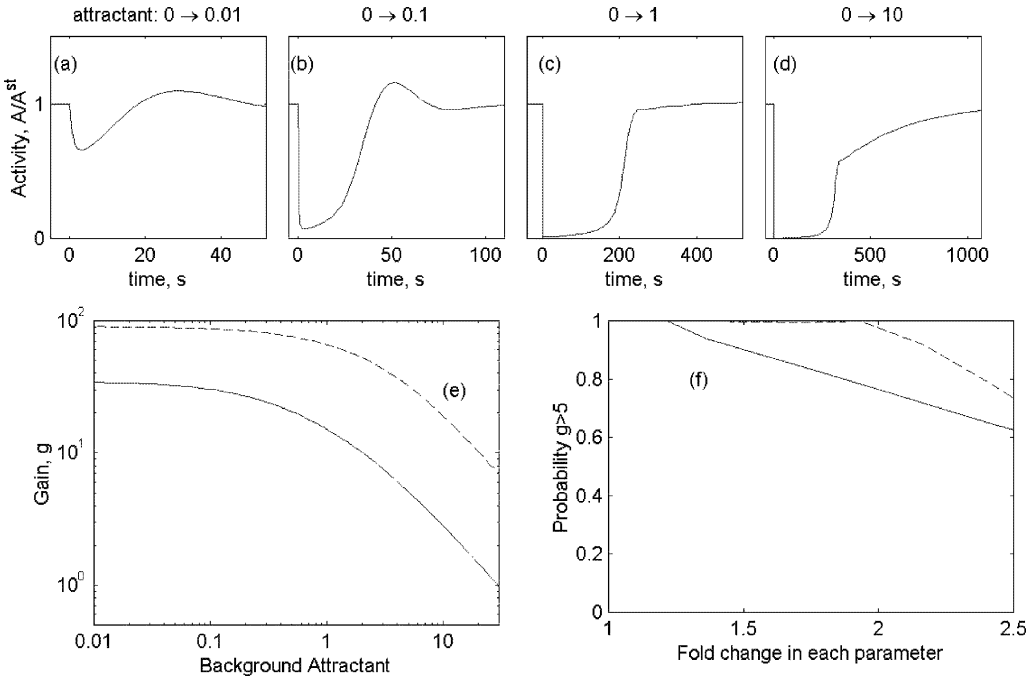

Figure 3. Amplified response to a step-like change in attractant concentration. (a)–(d) Kinetics of the relative system activity, $A/A^{\rm st}$, following a stimulation. The values of rate constants used are given in Section 2. The indicated amount of attractant ℓ, in units of the receptor dissociation constant, was added to a ligand-free system at time $t = 0$. Consistent with experiments, adaptation times vary strongly with the change in receptor occupancy, changing from a few seconds at small stimuli to several minutes at large stimuli [22,23]. After large stimulation, the return to steady state is not exponential and resembles experimentally observed kinetics [19,22,23]. Overshoot is observed, although its existence and extent depend on the model's parameters. (e) The gain as a function of background attractant ℓ_0, for a small stimulation $\ell_0 \to \ell_0 + \Delta\ell$, with $\Delta\ell/(1 + \ell_0) = 0.01$. (f) Robustness of amplification. The gain, g, to an addition of $\ell : 0 \to 0.01$, was calculated for 2000 model systems, obtained by randomly increasing or decreasing all the biochemical parameters of the system simulated in (a)–(d). The probability that g is larger than 5 is plotted as a function of the fold change in each parameter. Dashed line in (e)–(f): system with increased rate of CheB binding to receptors. Analoguous curves for the toy model (*figure 2*) show similar features.

to stimuli that change receptor occupancy by less than 1% [11,12]. Despite the detailed characterization of the chemotaxis network [13], the origin of this high gain is poorly understood [14]. Recently, it was suggested that amplification in chemotaxis is due to receptor clustering [3], or cooperative binding of P-CheY to the motor. Here we propose that the inhibition-driven amplification mechanism might be at work in the chemotaxis network. The main assumption is that the demethylating enzyme CheB functions also as the inhibitor I. A role for CheB in providing high gain was hinted at by the experimental observation that CheB mutants are severely deficient in amplification [11,15–18]. The present model, as applied to chemotaxis shows a large amplification while preserving exact adaptation (*figure 3a–d*). In accordance with experiments [11,12] a change in the receptor occupancy smaller than 1% elicits a detectable response. The system shows large dynamic range (*figure 3e*), by responding to small signals over several decades of background attractant concentration. The high gain of the system is a robust property: it remains significant despite variations in any of the network's biochemical parameters, *figure 3f*. We would like to stress that sensitivity amplification mechanism proposed by the present model is not exclusive, and in principle, several other mechanisms could contribute to the high gain. These may include cooperative interactions between the receptors and nonlinearity of the response of the flagellar motors.

The relevance of the inhibition-driven amplification mechanism to chemotaxis is yet to be verified experimentally. The model makes several predictions that could be used to experimentally disprove its relevance. It relies on a strong binding of CheB to active receptor complexes. This binding is in principle accessible to *in vitro* experiments. In addition, the model predicts that CheB mutants that cannot be phosphorylated would be deficient in sensitivity amplification. (Within the present model, the phosphorylation of CheB is essential. Effectively, it decouples the two distinct activities of CheB: demethylation and inhibition of the active receptor. Without phosphorylation, the strong binding of CheB to receptor would result in a constant rate of demethylation. Robust adaptation, however, requires an activity-dependent demethylation rate [7]. Phosphorylation of CheB, whose rate is proportional to the system activity, provides the feedback loop necessary for adaptation.)

If valid, the model may explain several puzzling features of chemotaxis. First, the phosphorylation of CheB does not seem to be required for exact adaptation [19] and its role has been unclear. The model implies this phosphorylation feedback is essential for amplification. Second, in the present model, exact adaptation is tightly connected with sensitivity amplification, which is a key feature for chemotaxis. It is thus possible that exact adaptation evolved together with sensitivity amplification. This may shed light on the experimental finding that exact adaptation is robust to large variation in protein concentrations [19].

Sensitivity amplification and wide dynamic range are often required for sensory transduction. The main implication of this work is that these two features can be reconciled within a single network and this without precise adjustment of its biochemical parameters. We presented a general mechanism based on a tightly binding inhibitor of signaling activity. It would be interesting to explore whether it is an example of a broader class of robust sensitivity amplification mechanisms [20].

2. Material and methods

2.1. Derivation of the gain, equation (1)

We consider the response of the system to a $\Delta\ell$ change in ligand on intermediate time scales, slow compare to the conformation changes and inhibitor binding reactions but fast compared to the modification reactions of R and B (*figure 2*). On these time scales, the total number of modified receptors, $E_m^{\rm tot} = E_m + E_m^* + \{E_m^* I\}$, is approximately constant. To estimate the gain, consider a system in which the inactive state is favored, $\alpha^+ < \alpha^-, \beta$, and where binding of ligand stabilizes the inactive state, $\alpha^+ \propto 1/(1+\ell)$. Upon addition of ligand, the activity $A = E_m^*$ and the level of the inhibited complex $\{E^* I\}$ will decrease by ΔA and $\Delta\{E_m^* I\}$, respectively. The steady state condition, $0 = {\rm d}E_m/{\rm d}t \approx \alpha^- E_m^* + \beta\{E_m^* I\} - \alpha^+ E_m$, can be written as: $(\alpha^- + \alpha^+)E_m^* + (\beta + \alpha^+)\{E_m^* I\} = \alpha^+ E_m^{\rm tot}$. Comparing this intermediate-time steady state before and after the addition of ligand, we obtain

$$\frac{\Delta A + r\Delta\{E_m^* I\}}{A^{\rm st} + r\{E_m^* I\}} = \frac{\Delta\ell}{1+\ell} \quad \text{with} \quad r = \beta/\alpha^-$$

The gain is large when $\Delta\{E^* I\} \ll \Delta A$. In particular, when the binding of inhibitor is very strong, $\{E_m^* I\} = I_{\rm tot}$ and $\Delta\{E_m^* I\} = 0$, leading to the gain

$$g = \frac{A^{\rm st} + r\{E_m^* I\}}{A^{\rm st}} \approx r\frac{I_{\rm tot}}{A^{\rm st}}$$

Note that we focus on the case where ligand binding shifts the equilibrium toward the inactive receptor state. In the case where ligand binding promotes the active state, the appropriate definition of the gain would be the ratio between the fractional change in output and the fractional change in *occupied* receptors. In the context of chemotaxis, gain was previously defined according to the change in receptor occupancy [3,11, 12]. The gain according to the latter definition is higher at high attractant backgrounds.

2.2. Inhibition-driven amplification model for bacterial chemotaxis

Chemotactic attractants are sensed by a receptor, which forms a complex with a kinase CheA and an adaptor molecule CheW [17]. This complex is considered here as a single entity and is assumed to have two functional states, an *active* state, E^*, and an *inactive* state E [7]. In the active state, the kinase CheA can phosphorylate the two response regulators, CheB (B) and CheY. The receptor is also subject to reversible methylation. E^*_m and E_m denote the active and inactive receptor complexes that are methylated on $m = 0, \ldots, M$ residues. Methyl groups are added by CheR (R), and removed by P-CheB (B_p). The forward and backward transition rates from E_m to E^*_m, α^+_m and α^-_m, depend on the ligand concentration, ℓ. The output of the network is the concentration of active receptors, $A = \sum_m E^*_m$. This output is related to the concentration of P-CheY, which binds to the flagellar motors, inducing a change in swimming direction. The amplification mechanism relies on the assumption that the complexes of active receptor and CheB or P-CheB ($\{E^*_m B\}$, $\{E^*_m B_p\}$) have no kinase activity. These complexes can dissociate, releasing an inactive receptor, with a rate β_m. For simplicity, phosphorylation of CheB is assumed to be a first order reaction with the rate $k_+ A$. P-CheB is dephosphorylated at rate k_-. The model thus consists of the following reactions:

$$E^*_m \underset{\alpha^+_m(\ell)}{\overset{\alpha^-_m(\ell)}{\rightleftharpoons}} E_m$$

$$E^*_m + B_p \underset{d_{bp}}{\overset{a_{bp}}{\rightleftharpoons}} \{E^*_m B_p\} \xrightarrow{k_{bp}} E^*_{m-1} + B_p$$

$$E^*_m + B \underset{d_b}{\overset{a_b}{\rightleftharpoons}} \{E^*_m B\} \xrightarrow{k_b} E^*_{m-1} + B$$

$$E_m + R \underset{d_r}{\overset{a_r}{\rightleftharpoons}} \{E_m R\} \xrightarrow{k_r} E_{m+1} + R$$

$$\{E^*_m B\} \xrightarrow{\beta_m(\ell)} E_m + B$$

$$\{E^*_m B_p\} \xrightarrow{\beta_m(\ell)} E_m + B_p$$

$$B \underset{k_-}{\overset{k_+ A}{\rightleftharpoons}} B_p$$

where the activity $A = \sum_m E^*_m$. These reactions were represented in the standard way by a set of mass action differential equations. For instance, the kinetic equations for E^*_m and B are:

$$\frac{\mathrm{d}E^*_m}{\mathrm{d}t} = \alpha^+_m E_m - \alpha^-_m E^*_m + (1-\delta_{m,0})\big[-a_b E^*_m \cdot B - a_{bp} E^*_m \cdot B_p + d_b\{E^*_m B\} + d_{bp}\{E^*_m B_p\}\big] + (1-\delta_{m,M})\big[k_b\{E^*_{m+1} B\} + k_{bp}\{E^*_{m+1} B_p\}\big]$$

$$\frac{\mathrm{d}B}{\mathrm{d}t} = k_- B_p - k_+ A \cdot B + \sum_{m=1}^{M} \big[(k_b + d_b + \beta_m)\{E^*_m B\} - a_b E^*_m \cdot B\big]$$

The assumption that CheB phosphorylation follows linear kinetics can be relaxed. Note that we assume that CheB has two distinct ways to interact with the active receptor complex. For instance, CheB can bind to two different sites: one at which it can be phosphorylated by CheA, and second from which it can demethylate the receptor. The latter binding would also inhibit the ability of CheA to phosphorylate CheY and CheB.

In *E. coli*, several types of chemoreceptors signal through the same phosphorylation cascade. Signaling via any of the receptors will be amplified by the present mechanism. This may explain chemotaxis to

attractants that act through low abundance receptors. Within the present model, repellent stimuli are also amplified, with the fractional change in the total number of occupied receptors as the relevant input.

2.3. Numerical simulation of the model

The simulated reference system (*figure 3*) had $M = 2$ methylation sites ($m = 0, 1, 2$) and the following parameters: $a_r = 0.2\ \mathrm{s}^{-1}\cdot\ \mu\mathrm{M}^{-1}$, $d_r = 0.1\ \mathrm{s}^{-1}$ and $k_r = 0.1\ \mathrm{s}^{-1}$, $a_b = 1\ \mathrm{s}^{-1}\cdot\mathrm{nM}^{-1}$, $d_b = 1\ \mathrm{s}^{-1}$, $k_b = 0$, $a_{bp} = 0.1\ \mathrm{s}^{-1}\cdot\mathrm{nM}^{-1}$, $d_{bp} = 0.01\ \mathrm{s}^{-1}$, $k_{bp} = 1\ \mathrm{s}^{-1}$, $k_+ = 1\ \mathrm{s}^{-1}\cdot\ \mu\mathrm{M}^{-1}$, $k_- = 1\ \mathrm{s}^{-1}$, $\alpha_0^+ = 10\ \mathrm{s}^{-1}$, $\alpha_1^+ = 1/(1+\ell)\ \mathrm{s}^{-1}$, $\alpha_2^+ = 0$, $\alpha_0^- = 0$, $\alpha_1^- = \ell/(1+\ell)\ \mathrm{s}^{-1}$, $\alpha_2^- = 10\ \mathrm{s}^{-1}$, $\beta_0 = 0$, $\beta_1 = 2.5\ell/(1+\ell)\ \mathrm{s}^{-1}$, and $\beta_2 = 25\ \mathrm{s}^{-1}$. The concentration of receptors, CheR and CheB are 10 μM, 0.2 μM and 2 μM, respectively. These parameters were chosen in the range of experimental data where available, though most parameters were not measured. Clustering of the receptors at the cell pole might effectively increase the concentration of CheB and receptors and lead to high binding rates. To consider this possibility, we also used a system with reference parameters, except for $a_b = 100\ \mathrm{s}^{-1}\cdot\mathrm{nM}^{-1}$, $a_{bp} = 10\ \mathrm{s}^{-1}\cdot\mathrm{nM}^{-1}$, $k_+ = 10\ \mathrm{s}^{-1}\cdot\ \mu\mathrm{M}^{-1}$, $k_- = 1000\ \mathrm{s}^{-1}$ (dashed lines in *figure 3e–f*). This system has a higher binding rate of CheB to the receptor resulting in increased gain and robustness. Note that available data on the in vitro affinity of CheB for CheA (in the μM range) was obtained in the absence of active receptor complexes [21]. Numerical solutions were obtained using the ode23t routine of MATLAB 5.2 (Mathworks), executed on a PC.

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