The Control of the Controller: Molecular Mechanisms for Robust Perfect Adaptation and Temperature Compensation

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ABSTRACT Organisms have the property to adapt to a changing environment and keep certain components within a cell regulated at the same level (homeostasis). “Perfect adaptation” describes an organism’s response to an external stepwise perturbation by regulating some of its variables/components precisely to their original preperturbation values. Numerous examples of perfect adaptation/homeostasis have been found, as for example, in bacterial chemotaxis, photoreceptor responses, MAP kinase activities, or in metal-ion homeostasis. Two concepts have evolved to explain how perfect adaptation may be understood: In one approach (robust perfect adaptation), the adaptation is a network property, which is mostly, but not entirely, independent of rate constant values; in the other approach (nonrobust perfect adaptation), a fine-tuning of rate constant values is needed. Here we identify two classes of robust molecular homeostatic mechanisms, which compensate for environmental variations in a controlled variable’s inflow or outflow fluxes, and allow for the presence of robust temperature compensation. These two classes of homeostatic mechanisms arise due to the fact that concentrations must have positive values. We show that the concept of integral control (or integral feedback), which leads to robust homeostasis, is associated with a control species that has to work under zero-order flux conditions and does not necessarily require the presence of a physico-chemical feedback structure. There are interesting links between the two identified classes of homeostatic mechanisms and molecular mechanisms found in mammalian iron and calcium homeostasis, indicating that homeostatic mechanisms may underlie similar molecular control structures.

INTRODUCTION

Many physiologically important compounds are under tight homeostatic regulation, where internal concentrations are adapted (1) at certain levels, despite environmental disturbances. Two concepts have developed to understand homeostasis: one is related to the intrinsic properties of the network showing that the adaptation response is independent of (most but not all) rate constant values (referred to here as robust (2–4) adaptation/homeostasis), whereas the other concept looks at the homeostasis due to a fine-tuning between rate constants. Perfect adaptation describes an organism’s response to an external stepwise perturbation by regulating some of its variables/components precisely to their original preperturbation values. Perfect adaptation has been found, for example, in bacterial chemotaxis (5–8), photoreceptor responses (9), and MAP-kinase regulation (10–12). In this respect, perfect adaptation and homeostasis are closely related and in the following, we look at homeostasis as a perfectly adapted process.

Robust perfect adaptation/homeostasis of a perturbed system can be related to the concept of integral control (13) or integral feedback (14). In this type of control mechanism, the error between the value of the system output (controlled variable, CV) and its setpoint is integrated, and the integral value is fed to the input of the process (the so-called manipulated variable, MV), which results in a robust adaptation of the system output to the setpoint (Fig. 1). Recently, El-Samad et al. (15) have shown that calcium homeostasis under hypo-
calcemia conditions can be described on the basis of an integral feedback approach, where the error between the calcium setpoint and the actual calcium level is related to the activity of the parathyroid hormone (PTH), an important hormone in calcium regulation.

However, the molecular mechanisms behind error-sensing processes are little understood. To investigate the relationship between the integral control/feedback concept and its reaction kinetic realization, we provide here a kinetic analysis. We show that robust perfect adaptation (homeostasis) is associated with a control species working under zero-order flux conditions while acting on another control species in the way of a “control of the controller”. There is an interesting and close analogy between the mechanisms shown here and mechanisms found in mammalian iron and calcium homeostasis, indicating that other homeostatic mechanisms may underlie similar control structures.

Computational methods

Rate equations were solved numerically by using the FORTRAN subroutine LSODE (Livermore Solver of Ordinary Differential Equations) (16) and MATLAB (www.mathworks.com). To make notations simpler, concentrations are denoted by their names without square brackets.

RESULTS

Molecular representation of integral control

Fig. 2 a shows a simple scheme where a homeostatic-regulated intermediate A is being synthesized, transformed, and
degraded. Rate constant $k_{\text{pert}}$ indicates an environmental perturbation, such as a sudden increase in $A$. To avoid possible cell damage by excess of $A$, $A$ has to be homeostatically regulated. A way to achieve this is to use an $A$-induced enzyme, $E_{\text{adapt}}$, which clears the cell for excess $A$. To make the homeostasis perfect, i.e., $A$ adapts always to the same $A_{\text{set}}$ value, the rate in the formation of $E_{\text{adapt}}$ has to be proportional to the difference (i.e., the error) between $A$ and its setpoint, $A_{\text{set}}$, as indicated by the following equations and shown in Fig. 2, $a$ and $b$:

$$
\frac{dA}{dt} = k_{\text{synth}} + k_{\text{pert}} - \frac{V_{E_{\text{max}}} A}{K_{E_{\text{M}}} + A} - \frac{V_{E_{\text{max}}} A}{K_{E_{\text{E}}} + A},
$$

(1)

$$
\frac{dE_{\text{adapt}}}{dt} = k_{\text{adapt}}(A - A_{\text{set}}).
$$

(2)

However, writing the rate of formation of $E_{\text{adapt}}$ in proportion to the error $(A - A_{\text{set}})$ (Eqs. 1 and 2, and Fig. 2 $c$) does still lack a molecular understanding of how the setpoint $A_{\text{set}}$ is determined. In addition, treating the setpoint $A_{\text{set}}$ as a fixed parameter can lead to the problem that, for certain parameter values, concentrations in $E_{\text{adapt}}$ may become negative (Fig. 2 $b$).

To avoid negative concentrations, the zero-order term in Eq. 2, $j_0 = k_{\text{adapt}}A_{\text{set}}$, has to be replaced in a kinetically plausible way. A possibility is the removal of $E_{\text{adapt}}$ by an additional controller/enzymatic species ($E_{\text{set}}$) working at zero-order conditions. In this case, the set-value $A_{\text{set}}$ is then determined by $E_{\text{set}}$’s maximum velocity, $V_{E_{\text{max}}}$, divided by the $A$-induced influx rate, which generates $E_{\text{adapt}}$ (Eq. 3). Fig. 3 shows two representations of this mechanism with robust perfect adaptation/homeostasis in $A$ avoiding any negative concentrations. In Fig. 3 $a$, a fully expanded Michaelis-Menten mechanism is shown, whereas in Fig. 3 $b$ the mechanism is formulated in terms of steady-state or rapid equilibrium assumptions for the individual enzymatic steps. The kinetic equations with rate constants are given in the Appendix. For both cases, the setpoint in $A$ is given by

$$A_{\text{set}} = \frac{V_{E_{\text{max}}}^{E_{\text{set}}}}{k_{\text{adapt}}} = \frac{k_{\text{adapt}}E_{\text{set}}^{E_{\text{set}}}}{k_{\text{adapt}}},
$$

(3)

where $E_{\text{set}}^{E_{\text{set}}}$ is the total concentration of enzyme $E_{\text{set}}$. Keeping $A_{\text{set}}$ fixed, the mechanism shows robust homeostasis in $A$ even when rate constants of the three enzymatic pathways (Fig. 3 $a$) are varied by over six orders of magnitude! Fig. 4 shows the $A$-homeostasis for the scheme shown in Fig. 3 $a$, using several perturbing and initial conditions (for details, see Appendix and Fig. 3 legend). Fig. 4 $a$ shows the homeostasis in $A$ when $k_{\text{pert}}$ is increased from 0.0 to 1.0 a.u. In Fig. 4 $b$, a large positive excursion in $A$ is observed when $k_{\text{pert}}$ is increased from 1.0 to $1 \times 10^3$ a.u., which is accompanied by an increased relaxation time in $A$ for reaching $A_{\text{set}}$. Negative excursions in $A$ are observed when $k_{\text{pert}}$ is decreased. This is illustrated in Fig. 4 $c$ when $k_{\text{pert}}$ is decreased from 1.0 to $1 \times 10^{-3}$ a.u.

However, due to the introduction of enzymatic zero-order kinetics (for avoiding negative concentrations in $E_{\text{adapt}}$), both mechanisms in Fig. 3 show homeostasis only for perturbations, which result in increased or moderate decreased levels in $A$. When a perturbation removes $A$ too quickly, then the homeostasis in $A$ breaks down. We therefore call this type
of homeostatic control for inflow-homeostatic control. In Fig. 4 a such a breakdown in A-homeostasis is illustrated by the A steady-state level ($A_{ss}$) in relation to the (total) concentration of the A-removing enzyme $E_{ir}$. When the removal rate of A becomes greater than the total production rate ($k_{syn} + k_{pen}$), $A_{ss}$ decreases below $A_{set}$ and homeostasis in A is lost. This type of homeostatic failure can be avoided by using controllers, which specifically address the removal of A (outflow-homeostatic control). A mechanism for calcium homeostasis under outflow conditions (hypocalcemia) was recently suggested by El-Samad et al. (15), but in this mechanism, the problem of zero-order fluxes and their association with negative concentrations was not addressed. Specific examples of other inflow and outflow homeostatic mechanisms are discussed below. Fig. 4 e illustrates the breakdown in A-homeostasis when the kinetics in the removal of $E_{adapt}$ by $E_{set}$ is no longer zero-order. For sufficiently large $k_{f}^{E_{adapt}}$ values, the $K_M^{E_{adapt}}$ becomes much lower than $E_{adapt}$, ensuring zero-order kinetics in the removal of $E_{adapt}$ and leading to $A_{ss}$ values which are equal to $A_{set}$. For lower $k_{f}^{E_{adapt}}$ values, the $K_M^{E_{adapt}}$ increases and the zero-order kinetics in the removal of $E_{adapt}$ are eventually lost leading to $A_{ss}$ values lower than $A_{set}$ and to the loss in the homeostasis of A. As shown in Fig. 4 e, the $A_{ss}$ values under non-zero-order conditions also depend on $k_{pen}$. In Fig. 4 f, two A-time profiles are shown for two perturbations, one applied for zero-order conditions ($k_{f}^{E_{adapt}} = 10^{12}$, upper curve), and the other for non-zero-order conditions ($k_{f}^{E_{adapt}} = 10^6$, lower curve). In both cases, $k_{pen}$ is increased from 1.0 to 5.0 a.u. at $t = 5.0$ a.u. Clearly, zero-order kinetics in the removal of $E_{adapt}$ is required to ensure robust homeostasis in A.

**Robust perfect temperature compensation**

Temperature is an important environmental parameter, which influences each reaction step in a reaction kinetic network. Van’t Hoff’s rule states that the velocity of a chemical or biochemical process increases generally by a factor between 2 and 3 (the so-called $Q_{10}$) when the temperature is increased by 10°C. A $Q_{10}$ of 2 corresponds to an activation energy of ~50 kJ/mol (17). In general, the concentration of a chemical component, a flux within a kinetic network, or the period length of an oscillatory network, can show temperature compensation/adaptation near a given reference temperature, $T_{ref}$, when the following balancing equation, here written for the concentration in A, is satisfied (18–21):

$$\frac{d\ln A}{dT} = \frac{1}{RT} \sum_i C_i^A E_i.$$

Here $C_i^A = \frac{d\ln A}{d\ln k_i}$ is the control coefficient (22,23) describing how sensitive concentration A is with respect to variations to the network’s rate constants $k_i$. The values $R$, $T$, and $E_i$ describe the gas constant, the temperature (in Kelvin), and activation energy (in J/mol) of the process indexed by $i$, respectively. The balancing equation (Eq. 4) requires a fine-tuning between the control coefficients and activation energies. In general, the resulting temperature compensation in A is not robust, i.e., temperature compensation is only observed within a local region around $T_{ref}$ (see, for example, (24)). Considering the network in Fig. 3, we have 21 rate constants with associated activation energies, and in general, temperature compensation in A is given by Eq. 4 including all 21 terms.

However, this situation changes dramatically when one assumes that $E_{adapt}$ is removed by $E_{set}$ under saturating (zero-order kinetics) conditions and that $E_{set}$’s turnover is negligible compared to the other fluxes associated with $E_{set}$. In this case, most of the control coefficients become zero, except for two, which are related to the rate constants $k_{adapt}$ and $k_{cal}^{E_{adapt}}$. Together with the concentration of $E_{set}$, $k_{adapt}$

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**FIGURE 3** To avoid negative concentrations in $E_{adapt}$, $f_0$ (Fig. 2 a) is represented as an enzymatic zero-order process. (a) Fully expanded Michaelis-Menten mechanism. The rate equations together with rate constants are shown in the Appendix. To obtain robust homeostasis in A, $E_{set}$ removes active $E_{adapt}$ into an inactive form $E_{adapt}^*$ under zero-order conditions with $A_{set}$ given by Eq. 3. (b) Same mechanism as in panel a, but formulating the Michaelis-Menten mechanism under steady-state/rapid equilibrium conditions. Rate equations are given in the Appendix. A zip-archive containing MATLAB and Berkeley Madonna versions of the model shown in Fig. 3 A with instructions and annotation available in the Supporting Material.
and $k_{cat}^{pert}$ define the setpoint for $A$ (Eq. 3). Due to the concentration summation theorem (22, 25, 26),

$$\sum_i C_i^{cat} = 0.$$  (5)

$C_i^{cat}$ and $C_i^{pert}$ have the same magnitude but opposite signs. This indicates that the network can show robust temperature compensation in the level of $A$ when the activation energies for $k_{cat}^{adapt}$ and $k_{cat}^{pert}$ are equal. In fact, when all activation energies are equal, say each reaction step has an activation energy $E_a$, then all concentrations of the reaction intermediates in the network, $I_j$, become robust perfectly adapted, as seen by Eq. 6:

$$\frac{d \ln I_j}{d t} = \frac{1}{RT^2} \sum_i C_i^{cat} E_a = \frac{E_a}{RT^2} \sum_i C_i^{cat} = 0.$$  (6)

Fig. 5, a and b, shows this situation for 5°C and 100°C. When activation energies are different (except for the activation energies of $k_{cat}^{adapt}$ and $k_{cat}^{pert}$), then only $A$ shows robust temperature compensation, whereas the concentrations of the other intermediates are no longer invariant. This is indicated in Fig. 5, c and d, for temperature changes between 5°C and 100°C.

**DISCUSSION**

**Zero-order kinetics, integral feedback, and homeostatic breakdown**

Integral feedback (14) or integral control (13) is a concept from control theory assuring that the output (the CV, Fig. 1) of a perturbed process is kept at a certain setpoint by integrating the associated error $e$ such that $e$ approaches zero (Fig. 1).

To keep the level of $A$ homeostatic-regulated by integral control/feedback, the rate of formation of an additional species ($E_{adapt}$) has to be linked to the error, integrated, and then fed into the production rate of $A$. Integrated $A$ is subtracted from $A_{set}$ and the error $e$ is recalculated (Fig. 2 c). Essential for this approach is the definition of the error $e$ through Eq. 2, which provides the actual condition that $A$ approaches $A_{set}$ when the system’s steady state is reached. Critically in this respect is the kinetic interpretation of the...
term \(k_{\text{adapt}}A_{\text{set}}\). To avoid unrealistic situations such as negative concentrations (Fig. 2 b), the zero-order flux needs to be put into a proper mechanistic perspective. To achieve this, the mechanism shown in Fig. 3 includes an additional enzymatic species \(E_{\text{set}}\) leading to zero-order degradation/inactivation in \(E_{\text{adapt}}\). This step is essential to obtain robust homeostasis. It requires that the level of \(E_{\text{set}}\) is kept constant and that the ratio between \(k_{\text{adapt}}\) and \(k_{\text{set}}\) remains unchanged. The latter condition is similar to that found by Levchenko and Iglesias for a model of eukaryotic chemotaxis (27) and a model by Ingalls et al. for a fast excitation-slow inhibition mechanism (Fig. 12.7 in (28)), where activation and inhibition steps are simultaneously activated by a common environmental signal. It may be noted that such a control is, principally, still based on balancing. In our model (Fig. 3 a), the balancing between 21 components has been effectively reduced to three parameters, as indicated by Eq. 3.

Interestingly, the kinetic restriction that concentrations must be positive leads to the breakdown of homeostasis for the mechanism in Fig. 3 at high removal/outflow rates in \(A\). Whereas the homeostasis in Fig. 2 is robust for both high inflow and high outflow rates in \(A\) (leading sometimes to unrealistic negative concentrations in \(E_{\text{adapt}}\), the chemically realistic mechanism shown in Fig. 3 works only for (high) inflow and moderate outflow rates in \(A\). To address the situation of \(A\)-homeostasis at higher outflow rates (outflow-homeostasis), another homeostatic mechanism is necessary. Fig. 6 shows four motifs of homeostatic control mechanisms, two addressing inflow-homeostasis (Fig. 6, a and b) and two addressing outflow-homeostasis (Fig. 6, c and d). Each of these mechanisms work properly when the perturbing inflow and outflow conditions in \(A\) match their appropriate working conditions, but will fail otherwise, i.e., when total outflow in \(A\) becomes too large for an inflow-homeostatic controller or when total inflow in \(A\) becomes too large in an outflow-homeostatic controller. Thus, biochemical homeostasis will, in general, require at least two types of mechanisms, i.e., one addressing inflow-homeostasis and another addressing outflow-homeostasis.

Fig. 6 a shows an outline of the inflow-control mechanisms described in Fig. 3. The inflow-control mechanism in Fig. 6 b shows a related scheme suggested by Yi et al., including a zero order reaction step (14), where instead of the increased removal of \(A\) the formation of \(A\) is inhibited by a molecular feedback loop.

Fig. 6 c shows an outflow-homeostatic mechanisms closely related to the scheme by El-Samad et al. (15), but avoiding negative concentrations, as shown in their Fig. 8. In our Fig. 6 d, outflow homeostatic control is achieved by inhibiting the outflow of \(A\) through \(E_{\text{adapt}}\). In the Appendix we show kinetic representations of these four mechanisms.

Robust perfect adaptation can be related to the concept of integral control or integral feedback (13,14), which involves a negative feedback in the control-theoretic formulation of the system as indicated in Fig. 1 or Fig. 2 c. Although some schemes, as in Fig. 6, b and d, or in the literature (14,29), contain molecular feedback inhibitions (molecular negative feedbacks), the presence of robust perfect adaptation, i.e., the behavior of a control-theoretic negative feedback, does not necessarily require molecular negative feedbacks. An example of robust perfect adaptation with integral feedback behavior but without molecular feedback loops is given by a consecutive reaction such as \(\rightarrow A \rightarrow B \rightarrow\), where \(B\) (or the flux forming \(B\)) can show robust perfect adaptation for any stepwise change in the rate constant forming intermediate \(B\) (11,30,31), as long as \(A\) is formed by zero-order kinetics.
Thus, the essential part to get robust homeostasis in the mechanisms shown in Fig. 6, and as illustrated in Fig. 4, e and f, is the presence of the zero-order kinetic term (i.e., "control of the controller").

Possible regulation points in homeostatic mechanisms

Iron homeostasis

Iron is an essential element for all mammalian cells, but gets toxic when in excess. Special transport and regulatory processes are therefore needed to ensure iron homeostasis within the organism as a whole as well as in individual cells (32). Ferroportin (33) and hepcidin (34) have been suggested to be two key players in iron homeostasis. Ferroportin is an iron exporter, which transports iron from cells such as macrophages or intestinal or liver cells into the blood plasma. Hepcidin, a liver-produced hormone, is a negative regulator of iron absorption with antimicrobial properties, which itself is under homeostatic regulation. An interesting regulatory aspect, which relates to the models in Fig. 3, is that hepcidin binds to ferroportin and leads to its degradation in a similar way as $E_{\text{seg}}$ removes $E_{\text{adapt}}$. Considering the interaction between ferroportin and hepcidin, the mechanism in Fig. 3 suggests that under iron inflow conditions, hepcidin may serve as a setpoint controller for cell-internal iron concentrations with ferroportin having the role as $E_{\text{adapt}}$, i.e., removing iron (A) out of the cell. The binding between ferroportin ($E_{\text{adapt}}$) and hepcidin ($E_{\text{seg}}$), which leads to the degradation of ferroportin ($E_{\text{adapt}}$) (34), may thus provide a mechanism of how hepcidin acts as a "control of the controller" and leads to potential robust homeostasis. Hepcidin works at concentrations as low as 10 nM (34) and can efficiently reduce upregulated ferroportin levels when iron influx into the cell is high (35). It is not known whether the removal of ferroportin by hepcidin at normal iron concentrations is a zero-order process.

Calcium homeostasis

Fig. 7 shows a scheme of calcium homeostasis in humans. Calcitonin (CT), parathyroid hormone (PTH), and the active form of vitamin D (calcitriol) are important (but not the only) factors involved in the regulation of Ca\(^{2+}\) and bone metabolism (36). PTH increases bone resorption and plasma Ca\(^{2+}\) levels. Calcitriol increases intestinal Ca\(^{2+}\) absorption, bone resorption, and plasma Ca\(^{2+}\). Calcitonin (CT) decreases bone resorption and plasma Ca\(^{2+}\). CaSR denotes the calcium-sensing receptor in the nephron, which appears to mediate effects of hypercalcemia on calcium excretion (37). In case of low calcium levels or when the outflow of calcium needs to be compensated for, an outflow-control mechanism like that indicated in Fig. 6 c may come into play. The mechanism is similar to that suggested by El-Samad et al. (15) for hypercalcemia. In this mechanism, $E_{\text{adapt}}$ plays the role of PTH. The level of PTH is decreased by increased calcium levels. Robust calcium homeostasis is obtained due to a zero kinetic formation rate of $E_{\text{adapt}}$ (PTH) and its downregulation by calcium. In the case of high calcium levels, an inflow-control mechanism like that shown in Fig. 6 a appears to be operative. High calcium (A) levels activate CT and CaSR, which are
biophysical levels quickly increased, reaching levels at 30 nmol/L at 40°C. Interestingly, the temperature compensation in the calcium homeostasis in the pachytene spermatocytes appears to be due to a balance between two opposing reactions, i.e., between uptake and leakage to and from the cell’s internal calcium stores, with determined activation energies of 62 kJ/mol and 55 kJ/mol, respectively.

Although robust homeostatic and adaptation mechanisms appear to be attractive concepts, it is still unclear to what extent temperature compensation (of oscillatory or nonoscillatory processes) is due to a balancing between individual reaction steps (18,21) or due to mechanisms as outlined in Fig. 5, where the balancing is reduced to a few parameters (39). Characteristic to all physiological and chemical temperature compensated systems (20,24,38,40–48) is that the compensation mechanism operates at a local (for the organism) important temperature range and not globally over the whole temperature range such as shown in Fig. 5. However, this does not necessarily invalidate homeostatic control structures as those shown in Figs. 3 and 6. The controllers (for example, $E_{\text{adapt}}$ and $E_{\text{set}}$) have to be seen in the context of the dynamics of the whole cell and the whole organism (2), a systems (biology) perspective (49–51), where the controllers themselves are controlled and influenced by factors important for other cellular purposes.

**APPENDIX**

**Rate equations, rate constants, and activation energies for the mechanism in Fig. 3 a.**

*Rate equations*

\[
\frac{dA}{dt} = k_{\text{pert}} + k_{\text{synth}} - k_{\text{cat}}^{E_{\text{adapt}}} AE_{\text{set}} - k_{\text{pert}}^{E_{\text{adapt}}} AE_{\text{adapt}}
+ k_{\text{pert}}^{E_{\text{adapt}}} (E_{\text{adapt}} \cdot A) + k_{\text{cat}}^{E_{\text{adapt}}} (E_{\text{set}} \cdot A)
\tag{7}
\]

\[
\frac{dE_{\text{adapt}}}{dt} = k_{\text{adapt}} A - k_{\text{pert}}^{E_{\text{adapt}}} AE_{\text{adapt}}
+ \left( k_{\text{cat}}^{E_{\text{adapt}}} + k_{\text{cat}}^{E_{\text{adapt}}} \right) (E_{\text{adapt}} \cdot A)
- k_{\text{pert}}^{E_{\text{adapt}}} E_{\text{set}} E_{\text{adapt}} + k_{\text{pert}}^{E_{\text{adapt}}} (E_{\text{adapt}} \cdot E_{\text{set}}),
\tag{8}
\]

\[
\frac{d(E_{\text{adapt}} \cdot A)}{dt} = k_{\text{pert}}^{E_{\text{adapt}}} A E_{\text{adapt}} - \left( k_{\text{cat}}^{E_{\text{adapt}}} + k_{\text{cat}}^{E_{\text{adapt}}} \right) (E_{\text{adapt}} \cdot A),
\tag{9}
\]

\[
\frac{dP}{dt} = k_{\text{cat}}^{E_{\text{adapt}}} (E_{\text{adapt}} \cdot A) - k_{\text{pert}}^{P} P,
\tag{10}
\]

\[
\frac{dE_{\text{set}}}{dt} = k_{\text{pert}}^{E_{\text{set}}} - k_{\text{pert}}^{E_{\text{set}}} E_{\text{set}} E_{\text{adapt}}
+ \left( k_{\text{cat}}^{E_{\text{set}}} + k_{\text{cat}}^{E_{\text{set}}} \right) (E_{\text{adapt}} \cdot E_{\text{set}}) - k_{\text{pert}}^{E_{\text{set}}} E_{\text{set}},
\tag{11}
\]
rate constants and activation energies

The following rate constants with zero initial concentrations (both a.u.) have been used unless otherwise stated in the text. Rate constants refer to 25°C. The Arrhenius equation \( k = A \exp(-E/RT) \) has been used to calculate the rate constant \( k \), at other temperatures \( A \): preexponential factor, assumed to be temperature-independent; \( E \): activation energy; \( R \), gas constant; and \( T \), temperature in Kelvin). All rate constants are given in arbitrary units (a.u.):

\[
\begin{align*}
  k_{\text{pert}} &\geq 0 \ (70 \text{ kJ/mol}) ; \\
  k_E^{\text{adapt}} & = 4.0 \ (50 \text{ kJ/mol}) ; \\
  k_r^{\text{adapt}} & = 2.0 \ (40 \text{ kJ/mol}) ; \\
  k_{\text{cat}}^{\text{adapt}} & = 3.0 \ (80 \text{ kJ/mol}) ; \\
  k_{\text{adapt}} & = 3.0 \ (90 \text{ kJ/mol}) ; \\
  k^P & = 1.0 \ (80 \text{ kJ/mol}) ; \\
  k^q & = 1.0e-13 \ (70 \text{ kJ/mol}) ; \\
  k_{E_0}^{\text{adapt}} & = 1.0e-7 \ (50 \text{ kJ/mol}) ; \\
  k_{E_1}^{\text{adapt}} & = 1.0e+11 \ (70 \text{ kJ/mol}) ; \\
  k_{E_2}^{\text{adapt}} & = 1.0e+7 \ (60 \text{ kJ/mol}) ; \\
  k_{E_3}^{\text{adapt}} & = 6.0e+6 \ (90 \text{ kJ/mol}) ; \\
  k_{E_4}^{\text{adapt}} & = 1.0 \ (30 \text{ kJ/mol}) ; \\
  k_{\text{synth}} & = 3.0 \ (40 \text{ kJ/mol}) ; \\
  k_{E_0} & = 1.0 \ (50 \text{ kJ/mol}) ; \\
  k_{E_1} & = 5.0 \ (60 \text{ kJ/mol}) ;
\end{align*}
\]

Rate equations for the mechanism in Fig. 3b/Fig. 6a

\[
\frac{dA}{dt} = k_{\text{pert}} + k_{\text{synth}} - \frac{V_{E_0}^{\text{max}} A}{K_M^{E_0} + A} - \frac{V_{E_1}^{\text{max}} A}{K_M^{E_1} + A}
\]

(17)

\[
\frac{dE_{\text{adapt}}}{dt} = k_{\text{adapt}} A - \frac{V_{E_0}^{\text{max}} E_{\text{adapt}}}{K_M^{E_0} + E_{\text{adapt}}}
\]

(18)

Rate equations for the mechanism in Fig. 6b

\[
\frac{dA}{dt} = \left( k_{\text{synth}} + k_{\text{pert}} \right) - \frac{V_{E_0}^{\text{max}} A}{(K_M^{E_0} + A)}
\]

(19)

\[
\frac{dE_{\text{adapt}}}{dt} = k_{\text{adapt}} A - \frac{V_{E_0}^{\text{max}} E_{\text{adapt}}}{(K_M^{E_0} + E_{\text{adapt}})}
\]

(20)

Rate equations for the mechanism in Fig. 6c

\[
\frac{dA}{dt} = k_{\text{synth}} + k_{E_0} A - \frac{V_{E_0}^{\text{max}} A}{(K_M^{E_0} + A)}
\]

(21)

\[
\frac{dE_{\text{adapt}}}{dt} = j_0 - \frac{V_{E_0}^{\text{max}} E_{\text{adapt}} A}{(K_M^{E_0} + E_{\text{adapt}})}
\]

(22)

Rate equations for the mechanism in Fig. 6d

\[
\frac{dA}{dt} = k_{\text{synth}} - \frac{(V_{E_0}^{\text{max}} + k_{\text{pert}}) A}{(K_M^{E_0} + A)(K_M^{E_0} + E_{\text{adapt}})}
\]

(23)
\frac{dE_{\text{adapt}}}{dt} = j_0 - \frac{V_{E\text{max}}E_{\text{adapt}}A}{(K_{E\text{M}}^2 + E_{\text{adapt}})} \tag{24}

The following rate constants with zero initial concentrations (both a.u.)
give perfect homeostasis in A with $A_{\text{set}} = 1.0$ when varying $k_{\text{synth}}$ (20):
$k_{\text{synth}} = 1.0; V_{E\text{max}} = 10; K_{E\text{M}}^2 = 1.0; \text{ and } K_{E\text{set}}^{\text{adapt}} = 1.0; \text{ zero-order flux}$
$j_0 = 1.0; V_{E\text{max}} = 1.0; \text{ and } K_{E\text{M}}^2 = 1.0-6.$

**SUPPORTING MATERIAL**

Supporting Material files are available at http://www.biophysj.org/biophysj

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