FEBS Letters 414 (1997) 430–434 FEBS 19166

Quantification of information transfer via cellular signal transduction pathways

Boris N. Kholodenko[a,*], Jan B. Hoek[a], Hans V. Westerhoff[b,c], Guy C. Brown[d]

[a]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, 1020 Locust St., Philadelphia, PA 19107, USA
[b]Department of Microbial Physiology, Biocentrum, Free University, Amsterdam, The Netherlands
[c]E.C. Slater Institute, Biocentrum, University of Amsterdam, Amsterdam, The Netherlands
[d]Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, UK

Received 23 July 1997

Abstract A conceptual framework is developed for the quantitative analysis of signal transfer through cellular signal transduction pathways and networks. This approach is referred to as signal transfer analysis and is based on formalisms that were first developed for the analysis of metabolic networks. Signal transduction is quantified as the sensitivity, known as the response coefficient of a target (e.g. an ion channel or transcription factor) to a signal (e.g. a hormone, growth factor or neurotransmitter). This response coefficient is defined in terms of the fractional change in the activated target brought about by a small fractional change in the signal. Quantifying the signal transduction in this way makes it possible to prove that for an idealized signaling cascade without feedback loops, the total response equals the product of all the local response coefficients, one for each level of the cascade. We show under which conditions merely having more levels in a cascade can boost the sensitivity of a target to a signal. If a signal propagates to a target through two different routes, these routes contribute independently to the total response, provided there is no feedback from the target. This independence makes the behavior of signaling cascades different from that of metabolic pathways, where different branches are connected through Kirchhoff's law. The relations between the total response and the local kinetics at each level are given for a number of network structures, such as branched signaling pathways and pathways with feedback. The formalism introduced here may provide a general approach to quantify cellular information transfer.

Key words: Protein kinase and phosphatase; Sensitivity; Signal transfer analysis; Feedback loop

1. Introduction

During the past decade our knowledge of signal transduction pathways has proliferated almost explosively. However this knowledge has been almost exclusively qualitative. In order to fill this gap we have adapted a form of sensitivity analysis known as metabolic control analysis (see [1–4] for reviews) to quantify the signal transfer from a signal molecule to a cellular response via a signaling pathway or network. The signaling machinery may include receptors, G-proteins, adapter and docking proteins, protein kinases and phosphatases, phospholipases, ion channels, transcription factors, and any other protein, ion, or molecule (see, e.g. [5]). There are several quantitative aspects of signal transduction that are of interest, e.g. (A) How does a receptor at the top of a signal transduction cascade control the target activation state at the bottom of a cascade? (B) Why do signaling cascades generally have more than one level? (C) How does the signal transfer and its control depend on branches and feedback regulatory loops between various levels of a cascade network? (D) How do different signaling pathways interact?

In this paper we describe how the steady-state signal transfer can be quantified and show how the response of the cellular target arises from the local responses at each level of the cascade. We start with analysis of a linear pathway and then consider signaling pathways with more than one independent route of signal transfer to the target, and with more than one target. We analyze how the response of a single cascade level to the immediately preceding level depends on the kinetics of the components involved. Finally, we show how the cellular response changes when a signal transduction cascade feeds back on itself.

2. Fundamentals

A signaling pathway usually consists of a cascade of cycles, where each cycle consists of two or more interconvertible forms of a signaling intermediate (e.g. a phosphorylated and dephosphorylated protein), and one (or more) of these forms affects the interconversion of forms at the next level down the cascade. Fig. 1 depicts schematically the signal transfer from a signal (S) to a target via a cascade that may include a receptor (E_1), adapter proteins, G-proteins, protein kinases/phosphatases or other signaling molecules. The signal may be a hormone, growth factor, cytokine or neurotransmitter, and the cellular target process (T) may be a channel conductivity, rate of transcription or phosphorylation state of some protein at the other end of the pathway.

The control of the steady-state signal transfer from S to T is quantified as the steady-state fractional change in the level of the target (T) divided by the (very small) fractional change in the level of the signal (S):

$$R_S^T = \lim_{\Delta S \to 0}\left(\left(\frac{\Delta T}{T}\right)/\left(\frac{\Delta S}{S}\right)\right) = d\ln T/d\ln S. \quad (1)$$

R is the sensitivity, known as the response coefficient in metabolic control analysis. It is essentially equal to the % change in the level of the activated target caused by a 1% change in signal. A response coefficient greater than 1 means that (small) fractional changes in the signal are amplified by the factor R, i.e. there is relative amplification of the signal. A response coefficient of 1 means that a fractional change in the signal causes an equal fractional change in the target, whereas

*Corresponding author.
E-mail: kholode1@jeflin.tju.edu

PII S0014-5793(97)01018-1

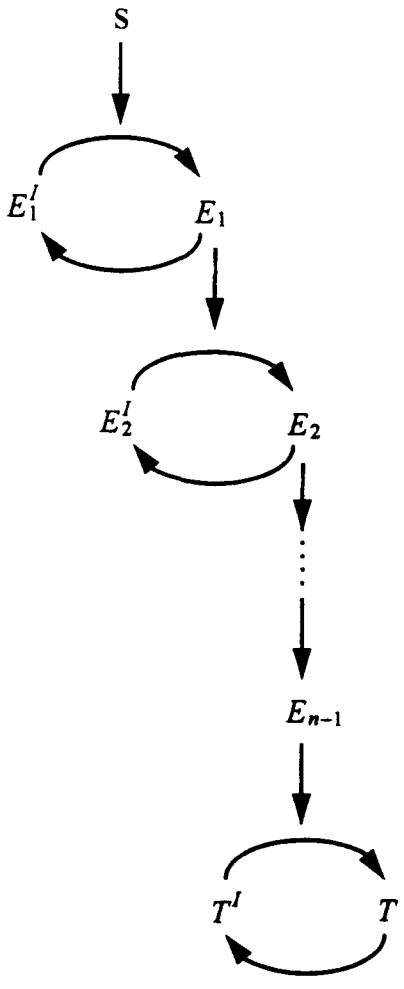

Fig. 1. A simplified scheme of a signal transfer through a signal transduction pathway. At each cascade level only two interconvertable forms are shown. Superscript I denotes inactive forms, e.g. E_1^I and E_1 stand for inactive and active receptor. The active form (E_i) at each level affects the activating and/or inactivating conversion at the subsequent level ($i+1$).

a response coefficient of less than 1 means that the fractional changes in the signal are diminished by the factor R. A response coefficient of 0 means that a change in signal has no effect on the target at a steady state, whereas a negative response coefficient means that an increase in signal causes a decrease in the target.

Within a cascade the response of any individual level (E_i) to the immediately preceding level (E_{i-1}) can be quantified as above, just as if there were a single level cascade:

$$r_{i-1}^{i} = \frac{d\ln E_i}{d\ln E_{i-1}}, \quad E_j = \text{const.} \tag{2}$$

Here the activities of proteins (signaling intermediates) at all other levels (j) are constant. We will call r_{i-1}^{i} the local response (local sensitivity) coefficient of level i to level $i-1$. This simply quantifies the relative amplification or suppression of a signal at this particular level of the cascade.

3. Signaling pathways without feedback

3.1. Linear pathways

For a linear cascade (Fig. 1), the response coefficient (i.e. the overall sensitivity) of a cascade is equal to the product of the local responses at each level of cascade (see Appendix, cf. [6]):

$$R_S^T = r_S^1 \cdot r_1^2 \cdot \ldots \cdot r_{n-1}^T = \Pi(\text{path}). \tag{3}$$

This equation is true where the signal and target are linked by a single linear cascade and there is no regulatory feedback or feedforward loops between cycles, i.e. one cycle cannot directly affect any cycle other than the next level down in the cascade (as in Fig. 1).

3.2. Branched pathways

If the signal and target are linked by more than one independent pathway (e.g. the signal has multiple receptors each with a different signal transduction pathway, Fig. 2A), then the total response is given by the sum of the products of local responses over each pathway:

$$R_S^T = \Pi(\text{path I}) + \Pi(\text{path II}). \tag{4}$$

Thus, the extent to which a signal controls a target through different pathways can be quantified and compared.

If the signal S affects two different targets, T_1 and T_2, through two signaling routes, which have a common part from S to X and at level X diverge into paths I and II (Fig. 2B), the responses of the targets are given by:

$$R_S^{T_1} = \Pi(\text{path } S \text{ to } X)\cdot\Pi(\text{path I}),$$
$$R_S^{T_2} = \Pi(\text{path } S \text{ to } X)\cdot\Pi(\text{path II}). \tag{5}$$

Therefore, changes in the targets brought about by changes in any pathway I or II, do not influence each other, unless a target feeds back on the signaling pathway before the branch point (see below).

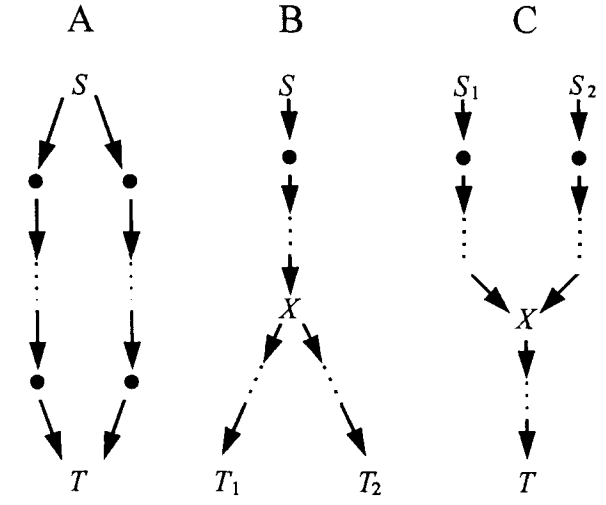

Fig. 2. Branched signal transduction pathways. Arrows show the influence of signaling molecule(s) at one level on the reactions at the subsequent level (the activating and inactivating interconversions at each level are not shown). A: Signal S affects two receptors with different signal transduction pathways leading to the same target T. B: The signal S affects two different targets, T_1 and T_2, through two signaling routes, which have a common part from S to X and diverge into paths I and II at level X. C: Two signals, S_1 and S_2, affect a target (T) through two signaling routes, which converge at X.

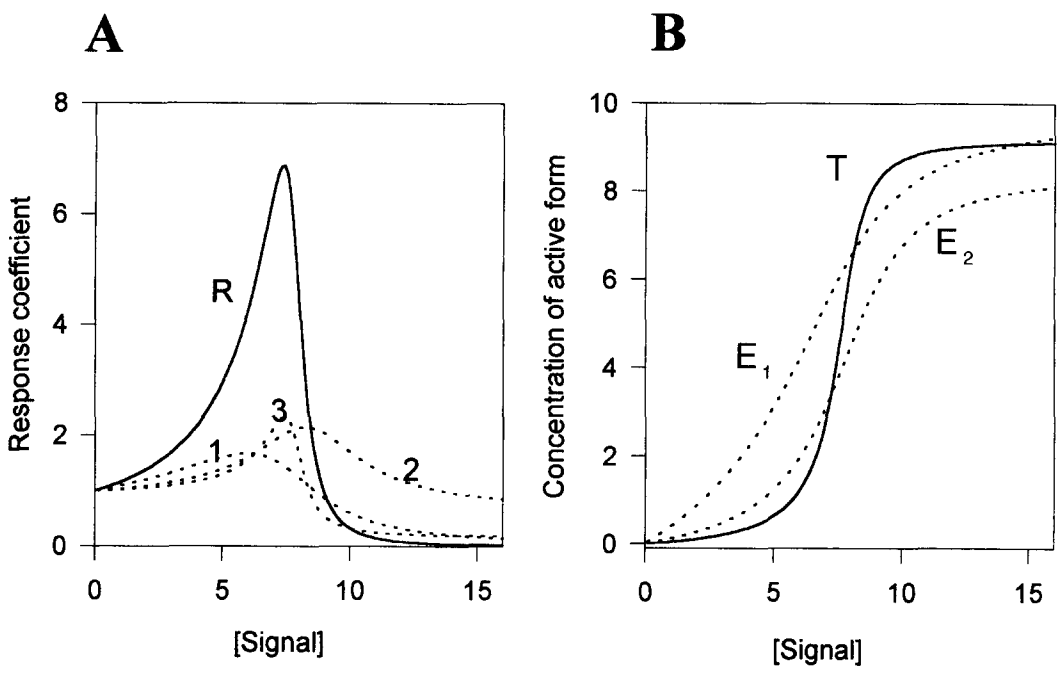

Fig. 3. Signal transduction via a three-level model cascade. A: Dependencies of the local and the total responses on the signal. Dashed lines 1, 2 and 3 correspond to the local responses. The total response of the target (R_S^T) is shown by the solid line. For simplicity, the kinases and phosphatases at each cascade level were assumed to follow Michaelis-Menten kinetics, $v_{Ai} = k_i^{cat} \cdot E_{i-1}/(1 + E_i^I/K_{mK}^i)$, $v_{Ii} = V_i^{max}/(1 + E_i/K_{mP}^i)$. The activity of the kinase at the first level was assumed to be proportional to the concentration of the signal, i.e. $E_0 = S$. The parameter values were (dimensionless units): $e_1 = (E_1 + E_1^I) = 10$; $k_1^{cat} = 1$, $K_{mK}^1 = 1$, $K_{mP}^1 = 4$, $V_1^{max} = 10$; $e_2 = (E_2 + E_2^I) = 10$; $k_2^{cat} = 1$, $K_{mK}^2 = 0.5$, $K_{mP}^2 = 3$, $V_2^{max} = 10$; $e_3 = T + T^I = 10$, $k_3^{cat} = 1.5$, $K_{mK}^3 = 1$, $K_{mP}^3 = 2$, $V_3^{max} = 10$. B: Increase in the sensitivity of the activation state to changes in the signal with the level of the cascade. The dependencies of the concentrations of E_1 (- - -), E_2 (- - -) and T (——) on S are shown.

If there are two signals, S_1 and S_2, affecting T through two signaling routes, which converge at X (Fig. 2C), the total response to equal relative changes in either signal is given by:

$$R_{12}^T = R_{S_1}^T + T_{S_2}^T = (\Pi(\text{path } S_1 \text{ to } X) + \Pi(\text{path } S_2 \text{ to } X)) \cdot \Pi(\text{path X to T}). \quad (6)$$

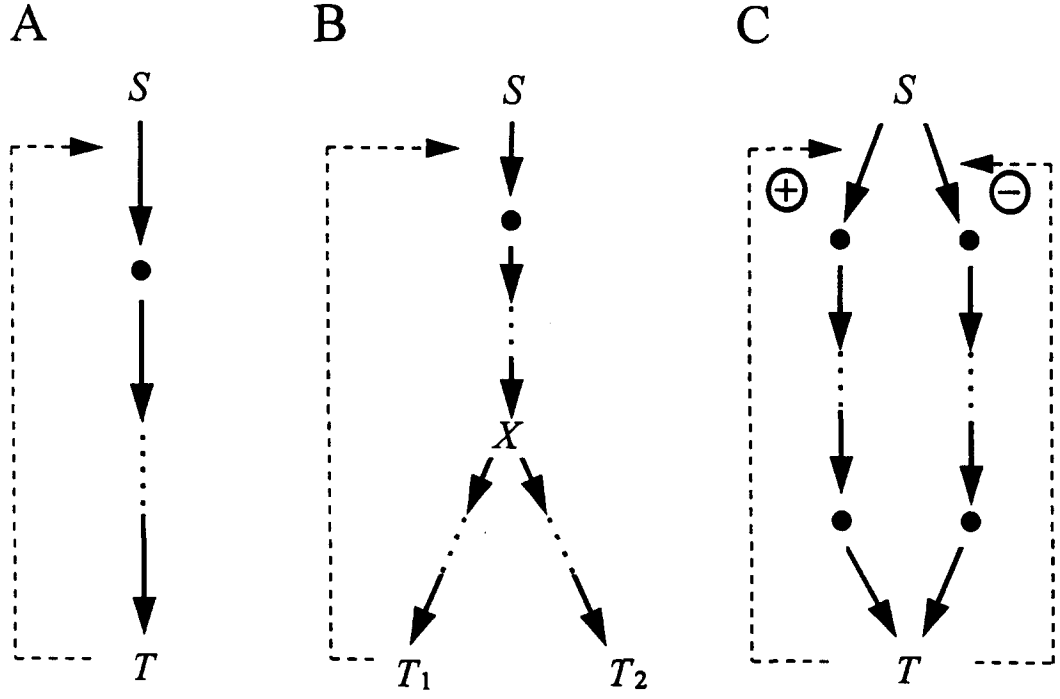

Fig. 4. Signal transduction pathways with feedbacks. A: A linear cascade that feeds back on itself. B: The branched signal transduction pathway of Fig. 2B with a feedback from a target (T) to the top of the pathway. C: The pathway of Fig. 2A, where the target (T) feeds back on one or both receptors of different signal transduction routes.

3.3. How does the local response depend on the kinetics of a particular level?

We shall consider a single cycle (i) of a cascade as if it operates in 'isolation' of the other cycles and estimate the local response coefficient, r^i_{i-1}, of cycle (i) to the preceding cycle ($i-1$). In the cycle of interconversion of a protein (or other signaling molecule) between the inactive (E^{I}_i) and active (E_i) form, the steady-state concentration, E_i, obeys the following kinetic equation:

$$\frac{\mathrm{d}E_i}{\mathrm{d}t} = v_{\mathrm{A}i} - v_{\mathrm{I}i}. \tag{7}$$

Here $v_{\mathrm{A}i}$ and $v_{\mathrm{I}i}$ are the rates of the activation and the inactivation conversions (e.g. the protein kinase and protein phosphatase reactions). By analogy to metabolic control analysis, we shall describe the kinetics of both $v_{\mathrm{A}i}$ and $v_{\mathrm{I}i}$ in terms of their 'local' sensitivities to the substrates and products (E^{I}_i, E_i) and to the effectors/catalysts (E_{i-1}). These sensitivities are called the elasticity coefficients [1–4] and defined as:

$$\kappa_X = \frac{\partial \ln v_{\mathrm{A}i}}{\partial \ln X}, \ \rho_X = \frac{\partial \ln v_{\mathrm{I}i}}{\partial \ln X}, \ X = (E^{\mathrm{I}}_i, E_i, E_{i-1}). \tag{8}$$

The partial derivatives are taken at constant activities of all other proteins but X. Using Eq. (11) and taking into account that the total concentration (e_i) of the interconverted protein, $E^{\mathrm{I}}_i + E_i = e_i$, remains constant, one can readily express the local response, r^i_{i-1}, in terms of kinetics of the ith cycle [7]:

$$r^i_{i-1} = (\kappa_{E_{i-1}} - \rho_{E_{i-1}}) / \left(\rho_{E_i} - \kappa_{E_i} + (E_i/E^{\mathrm{I}}_i)\cdot(\kappa_{E^{\mathrm{I}}_i} - \rho_{E^{\mathrm{I}}_i})\right). \tag{9}$$

When only the activation rate $v_{\mathrm{A}i}$ is influenced by E_{i-1}, and there is no influence on $v_{\mathrm{I}i}$, then in Eq. (9) $\rho_{E_{i-1}}$ must be zero, and the local response to a signal at level i is positive, provided $\kappa_{E_{i-1}}$ is positive. If E_{i-1} catalyzes the activation conversion (so that the rate $v_{\mathrm{A}i}$ depends linearly on E_{i-1}), Eq. (8) shows that $\kappa_{E_{i-1}} = 1$. Often, the rate $v_{\mathrm{A}i}$ (e.g. the kinase) may not depend explicitly on E_i, and the rate $v_{\mathrm{I}i}$ (the phosphatase) does not depend explicitly on E^{I}_i, then Eq. (9) simplifies to:

$$r^i_{i-1} = 1/\left(\rho_{E_i} + (E_i/E^{\mathrm{I}}_i)\cdot\kappa_{E^{\mathrm{I}}_i}\right). \tag{10}$$

If only the rate $v_{\mathrm{I}i}$ of the inactivation conversion is affected by E_{i-1}, so that E_{i-1} is the phosphatase, then the local response coefficient, r^i_{i-1}, has the opposite (negative) sign. The general analysis above shows that the total response of the target to the signal is positive, if the number of cascade levels with negative local responses is even.

When both protein kinase and phosphatase follow Michaelis-Menten kinetics and are far from equilibrium (so that their rates, $v_{\mathrm{A}i}$ and $v_{\mathrm{I}i}$, do not depend on the products), the magnitudes of the elasticities, ρ and κ are between 1 and 0 (see, e.g. [8]):

$$k_{E^{\mathrm{I}}_i} = 1/(1 + E^{\mathrm{I}}_i/K_{\mathrm{mK}}); \ \rho_{E_i} = 1/(1 + E_i/K_{\mathrm{mP}}).$$

Here K_{mK} and K_{mP} are the Michaelis constants of the protein kinase and phosphatase. If at any level both protein kinase and phosphatase are saturated with E^{I}_i and E_i, respectively, so that their elasticities κ and ρ are low, the local response r^i_{i-1} at this level, is high. For a monocyclic cascade this phenomenon is known as 'zero-order ultrasensitivity' [9,10]. In general, when the total concentration of interconverted enzyme (e) is more than twice the affinities of the inactive and active forms for the catalysts (i.e. $e_i/2 \geq K_{\mathrm{mK}}, K_{\mathrm{mP}}$), the local response (sensitivity of the level) follows a bell-shaped curve. Lines 1, 2 and 3 in Fig. 3A show these sensitivities for each level of a three-level cascade.

3.4. When can a signaling cascade operate as a switch?

Since the local responses at each level multiply to give the total response of a cascade, the activated target can change dramatically with a change in the signal enabling the cascade to operate almost as a switch. Fig. 3 illustrates this numerically for a model cascade of three levels. Fig. 3A shows how the local responses, r^i_{i-1}, of each cascade level (dashed lines 1, 2 and 3) and the total response (R) of the target (solid line) depend on the signal (S). Fig. 3B shows that the steepness of the dependence of the activation state on the signal increases with the number of cascade levels (E_1, E_2 and T). If the protein kinases and phosphatases are far from saturation, the local response coefficients can be less than 1, and signal changes are attenuated (as e.g. in the higher range of signal strength in Fig. 3A, where E^{I}_i become very low).

In a recent numerical simulation of the kinetics of the MAPK cascade, Huang and Ferrell [11] showed that this cascade has a very high steady-state sensitivity. In the experimental studies they showed that this sensitivity was equivalent to a Hill coefficient of 5. In a numerical model Huang and Ferrell [11] found a successive increase in the steepness of the plots of activated MAPK cascade components at successively lower levels of the cascade. This is explained by our result that the total sensitivity equals the product of the sensitivities at each level.

4. Pathways with feedbacks

We shall now describe the dependence of the overall response on the local responses for pathways with feedback loops. For any particular mechanism of the target influence on the receptor, the feedback can be described in terms of the local response (r^1_T) as follows:

$$r^1_T = \frac{\mathrm{d}\ln E_1}{\mathrm{d}\ln T}, \ E_j = \mathrm{const.}$$

In the case of activation of the receptor by the target, r^1_T is positive, and r^1_T is negative in the case of inhibition.

Feedback changes drastically the total response of a target to a signal. For a linear cascade that feeds back on itself (Fig. 4A), the total response to a signal is given by (see Appendix):

$$R^T_S = \Pi(\mathrm{path}) / (1 - (r^1_T/r^1_S)\cdot\Pi(\mathrm{path})). \tag{11}$$

As expected, a positive feedback amplifies the control exerted by a receptor on a target and a negative feedback decreases the control. Importantly, if the strength of the feedback exceeds a certain magnitude, the signal transduction pathway cannot operate at steady state. For instance, an increase in positive feedback to the magnitude at which the denominator in Eq. (11) is zero, results in the loss of the stability of a steady state and forces the signaling system to oscillate. Oscillations can also occur as a result of a negative feedback [12,13].

In the absence of feedback different pathway branches add independently to the total response. Feedback loops change this simple picture. Let us consider again a signal transduction

pathway with two branches, diverging at level X and leading to different targets, T_1 and T_2 (see Fig. 2B), but now with a feedback from a target T_1 to the top of the signaling pathway (Fig. 4B). Then, the responses of the targets are given by:

$$R_S^{T_1} = \Pi(\text{path } S \text{ to } X)\cdot\Pi(\text{path I})/\Big(1-(r^1_{T_1}/r^1_S)\cdot\Pi(\text{path } S \text{ to } X)\cdot\Pi(\text{path I})\Big),$$

$$R_S^{T_2} = \Pi(\text{path } S \text{ to } X)\cdot\Pi(\text{path II})/\Big(1-(r^1_{T_1}/r^1_S)\cdot\Pi(\text{path } S \text{ to } X)\cdot\Pi(\text{path I})\Big). \quad (12)$$

Thus, due to a feedback from one target (T_1) the other target (T_2) becomes sensitive through changes in T_1 and in the 'independent' branch (path I) leading to T_1.

If a signal has two receptors each with a different signal transduction pathway to one target, which feeds back on one or both receptors (Fig. 4C), then the total response to the signal is given by:

$$R_S^T = \frac{\Pi(\text{path I}) + \Pi(\text{path II})}{1-(r^1_T(\text{I})/r^1_S(\text{I}))\Pi(\text{path I})-(r^1_T(\text{II})/r^1_S(\text{II}))\Pi(\text{path II})}. \quad (13)$$

This equation shows that even when only one pathway has a feedback (e.g. $r^1_T(\text{II}) = 0$), the two pathways no longer add independently to the total response. Signal transduction by the two pathways is integrated by the feedback.

Theories of information transfer (such as information theory) have been developed previously for the analysis of the transfer of digital information, and thus are not appropriate for signal transduction pathways in living cells. The theory outlined here, which we call signal transfer analysis, is similar to the modular control analysis [6,14], but aimed explicitly at an analysis of the transfer of analogue information within and between cells.

Appendix

For a linear cascade route without feedbacks or feedforwards (Fig. 1), the signal S causes changes at each cascade level i only through changes in the active form, E_{i-1}, at the immediately preceding level $i-1$, provided one can neglect the sequestration of molecules of level i by the successive level $i+1$ (cf. [15]). Therefore, the changes in E_i do not depend on changes in the activities at any levels of the cascade other than level $i-1$. Applying the chain rule for calculating the derivative of T with respect to S one arrives at Eq. (3) of the main text.

For a signal transduction pathway with a feedback loop from the target T to the receptor level (Fig. 4A), we shall estimate the control exerted by the signal S on the activation state (E_i) of the protein at level i. After a change in S, the changes in the activation state E_1 at the first level are brought about by changes in the activities of S and T,

$$\mathrm{dln}E_1 = r^1_S\cdot\mathrm{dln}S + r^1_T\cdot\mathrm{dln}T. \quad (A1)$$

The changes in the activation state E_i at level i occur due to changes in the activity of E_{i-1} only (in particular the changes in T occur through changes in E_{n-1}), see Fig. 4A:

$$\mathrm{dln}E_i = r^i_{i-1}\cdot\mathrm{dln}E_{i-1},\ i = 2,3,\ldots,n-1,$$

$$\mathrm{dln}T = r^T_{n-1}\cdot\mathrm{dln}E_{n-1}. \quad (A2)$$

Using Eq. (A2) repeatedly, and substituting the result in Eq. (A1) one arrives at Eq. (11) of the main text.

References

[1] Fell, D.A. (1992) Biochem. J. 286, 313–330.
[2] Kholodenko, B.N. and Westerhoff, H.V. (1995) TIBS 20, 52–54.
[3] Brand, M.D. (1996) J. Theor. Biol. 182, 351–360.
[4] Brown, G.C., Westerhoff, H.V. and Kholodenko, B.N. (1996) J. Theor. Biol. 182, 389–396.
[5] Amsterdam Special Issue of FEBS Letters (1997), v. 410, Number 1.
[6] Kahn, D. and Westerhoff, H.V. (1991) J. Theor. Biol. 153, 255–285.
[7] Small, J.R. and Fell, D.A. (1990) Eur. J. Biochem. 191, 405–411.
[8] Kholodenko, B.N. (1988) Mol. Biol. (USSR) 22, 1238–1256 (Engl. transl. 22 (1989) 990–1005).
[9] Goldbeter, A. and Koshland Jr., D.E. (1981) Proc. Natl. Acad. Sci. USA 78, 6840–6844.
[10] Koshland Jr., D.E., Goldbeter, A. and Stock, J.B. (1982) Science 217, 220–225.
[11] Huang, C.-Y.F. and Ferrell Jr., J.E. (1996) Proc. Natl. Acad. Sci. USA 93, 10078–10083.
[12] Tyson, J.J. and Othmer, H.G. (1978) Progr. Theor. Biol. 5, 1–60.
[13] Dibrov, B.F., Zhabotinsky, A.M. and Kholodenko, B.N. (1982) J. Math. Biol. 15, 51–63.
[14] Westerhoff, H.V., Koster, J.G., Van Workum, M. and Rudd, K.E. (1990) In: A. Cornish-Bowden (Ed.), Control of Metabolic Processes, Plenum, New York, pp. 399–412.
[15] Kholodenko, B.N., Lyubarev, A.E. and Kurganov, B.I. (1992) Eur. J. Biochem. 210, 147–153.