Mathematical Models of Specificity in Cell Signaling

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ABSTRACT   Cellular signaling pathways transduce extracellular signals into appropriate responses. These pathways are typically interconnected to form networks, often with different pathways sharing similar or identical components. A consequence of this connectedness is the potential for cross talk, some of which may be undesirable. Indeed, experimental evidence indicates that cells have evolved insulating mechanisms to partially suppress "leaking" between pathways. Here we characterize mathematical models of simple signaling networks and obtain exact analytical expressions for two measures of cross talk called specificity and fidelity. The performance of several insulating mechanisms—combinatorial signaling, compartmentalization, the inhibition of one pathway by another, and the selective activation of scaffold proteins—is evaluated with respect to the trade-off between the specificity they provide and the constraints they place on the network. The effects of noise are also examined. The insights gained from this analysis are applied to understanding specificity in the yeast mating and invasive growth MAP kinase signaling network.

INTRODUCTION

The proper growth, development, and survival of an organism requires extensive communication between that organism's cells, and accurate sensing of external conditions. Accordingly, cells sense and respond to a wide variety of chemical and environmental stimuli. Many incoming signals, including hormones such as insulin and adrenalin, are first recognized by a cell surface receptor, and then transmitted to various locations inside the cell by a cascade of signaling proteins that comprise a "signal transduction", or "signaling", pathway (Fig. 1 *A*). In general, different stimuli trigger distinct cellular responses that are appropriate given the nature of the stimulus. For instance, liver cells respond to insulin by taking up sugar from the blood and storing it as glycogen, whereas they respond to adrenalin by releasing stored sugar into the blood.

Different signal transduction pathways are often interconnected to form larger networks. Elements(s) of one pathway may cross-regulate one or more components of another pathway, or multiple distinct pathways may share some of their components (Fig. 1 *B*). Such cross-regulation may enable the cell to integrate its overall response when receiving multiple stimuli, and can enable the network to exhibit complex behaviors (1–4). However, extensive interconnections increase the difficulty of maintaining specificity from signal to cellular response; that is, they increase the likelihood that (under certain circumstances) the activation of one pathway may result in the undesirable activation or inhibition of another pathway. Thus, cross-regulatory interconnections likely evolved hand-in-hand with insulating mechanisms that function to limit undesirable spillover. The problem of "signaling specificity" is to understand the mechanisms that have evolved to maintain specificity from signal to response and to limit "leaking" between pathways, despite extensive interconnections and component sharing (5–11).

One informative experimental system to study signaling specificity is found in baker's/brewer's yeast (*Saccharomyces cerevisiae*), where interconnected protein kinase cascades regulate two distinct biological endpoints: mating and filamentous invasive growth (12). These endpoints are triggered by distinct stimuli, leading to the differential activation of downstream mitogen-activated protein kinases (MAPKs), and induction of an appropriate set of target genes (Fig. 1 *C*). Mating is initiated by mating pheromone and results in induction of genes mediating the fusion of two haploid cells (13,14), whereas invasive growth is triggered by mechanical and nutrient cues and results in changes in cell shape and adhesiveness (15). Both pathways use the sequentially acting protein kinases Ste20^{MAP4K}, Ste11^{MAP3K}, and Ste7^{MAP2K} (16,17). However, mating pheromone stimulates the activation of both Fus3^{MAPK} and Kss1^{MAPK}, whereas only Kss1 is activated during invasive growth (18,19). Despite this extensive component sharing, mating and invasive growth are normally reasonably well insulated from one another: cells exposed to mating pheromone do not initiate invasive growth, and cells growing invasively do not induce the mating program. However, mutations in certain key components of this signaling network can compromise specificity, so that treatment with pheromone leads to the induction of invasive growth genes, for example, (18,20–23). These findings, together with observations from other experimental systems, demonstrate that cells have evolved mechanisms that promote signaling specificity by limiting the extent of leaking
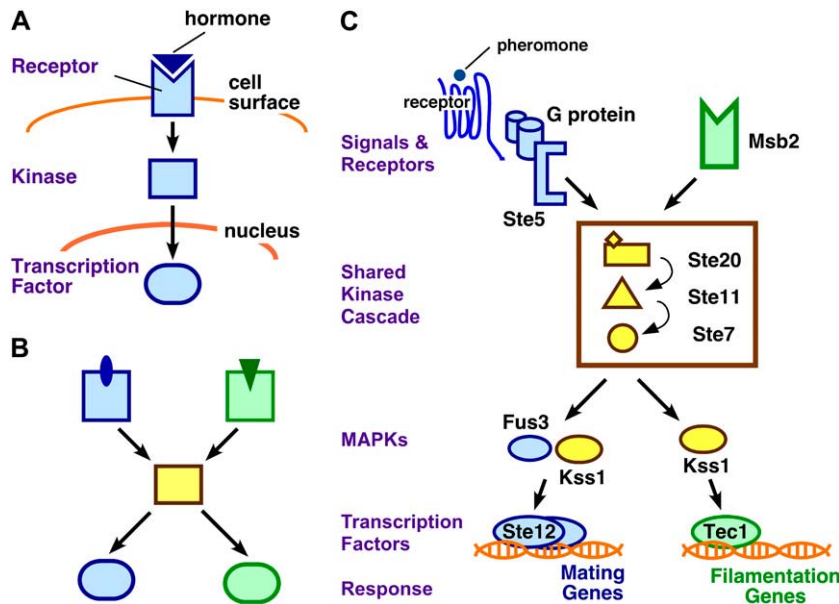
FIGURE 1 Signaling pathways and networks. (*A*) A cartoon of a simple signaling cascade. (*B*) A cartoon of a simple network with a shared component. (*C*) The yeast mating/invasive growth signaling network, which contains multiple shared components. Mating is initiated by the binding of peptide pheromone to a G-protein-coupled receptor, leading to the activation of a MAP kinase cascade and the induction of mating genes via homodimers of the Ste12 transcription factor. Invasive growth is triggered (in part) by signals transmitted by the Msb2 receptor via the MAP kinase cascade to the Tec1 transcription factor. Mating-pathway-specific components are colored blue, invasive growth-specific components are green, and shared components are yellow. See text for further details.

between pathways that share similar or identical components (7,8,12).

Because disruption of signaling specificity may play a role in the pathogenesis of cancer and other diseases (5,24–27), further understanding of mechanisms that promote specificity is warranted, both at the experimental and theoretical level. Here we extend our recently developed framework for the analysis of networks containing two or more signaling pathways (28). We concern ourselves with the following questions: How can the concept of specificity be precisely defined? Are there fundamental limits to specificity imposed by certain network architectures? How effective, in theory, are some of the insulating mechanisms found in nature that have been proposed to enhance specificity? Do certain insulating mechanisms impose additional constraints on the network? What is the effect of noise in stochastic signaling networks on specificity and fidelity? Finally, are there common features or emergent properties of signaling networks that exhibit a reasonable degree of specificity despite undesired signal crossover?

This article is organized as follows: first we describe a theoretical framework that allows us to reason about cell signaling and quantify signal specificity; we also give a mathematical description of very simple signaling networks and examine their specificity properties. Next we discuss several different insulating mechanisms, including combinatorial signaling, cross-pathway inhibition, and the action of a selectively activated scaffold protein. We present a mathematical analysis of simple networks employing each of these mechanisms and show how effective each mechanism is in increasing signal specificity. We also examine the effect of noise in stochastic signaling networks; we prove that the specificity and fidelity of linear and nonlinear networks is not affected by noise. Finally, we talk about the effect of back-

ground or basal output levels on specificity and fidelity and show how our definitions can be modified to include high background levels; this section also clarifies how specificity and fidelity can be measured experimentally. In the Discussion, some of the insights derived from our analysis are applied to the yeast mating/invasive growth network.

RESULTS

Description of the model

Fig. 2 *A* shows a simple signaling network composed of two signaling pathways, *X* and *Y*, initiated by signals $x_0(t)$ and $y_0(t)$, respectively. For pathway *X*, the time-dependent input signal, $x_0(t)$, activates (that is, causes the production of the active form of) the first component, x_1, which in turn activates the second component, and so on. The level of activation of the final component, x_f, is taken as a measure of pathway output (in Fig. 2 the final component is x_2), which determines the response of the cell to the signal: the cell may move, grow, divide, change its pattern of gene expression or its metabolism, etc.

The network shown in Fig. 2 is much simpler than real cellular signaling networks, which may consist of dozens of component proteins. However, a single tier in one of the abstract cascades may be taken to represent several successive steps in a real pathway. For instance, $x_0(t)$ may be taken to represent the plasma concentration of a hormone as a function of time, or the output of a subpathway consisting of hormone-receptor binding, the activation of a receptor-coupled G-protein, and several steps downstream of that. For convenience, we shall frequently call the first component of a pathway the signal, the intermediate component a kinase, and the final component the target.
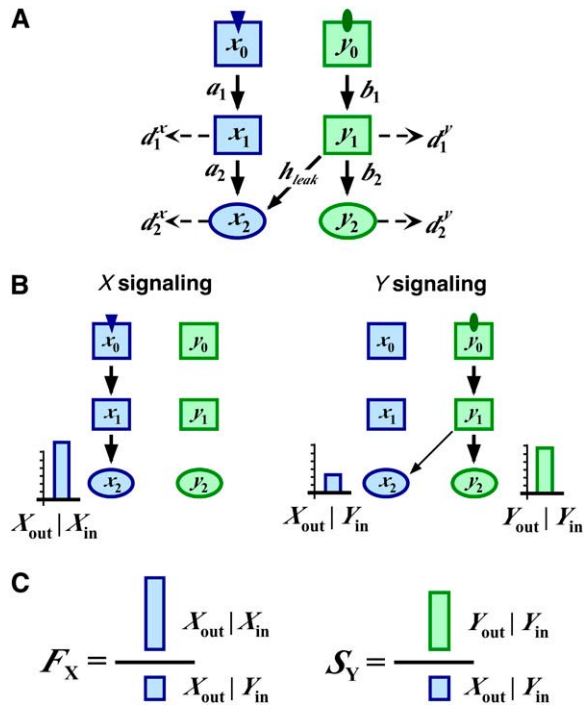
FIGURE 2 A simple network consisting of two parallel cascades with cross talk. The network consists of two pathways, X and Y. Pathway X has three components (x_0, x_1, and x_2); component x_0 is input signal itself, or is activated in manner that is strictly proportional to the input signal. (Because the input signal can have any shape, x_0 can abstractly represent any upstream component, e.g., a receptor, a G-protein, a kinase, etc.) The parameters a_1 and a_2 are activation rate constants, and d_1^x and d_2^x are deactivation, or decay, rate constants. For example, a_1 is the rate constant for the activation of x_1 by x_0, and d_1^x is the rate constant for the deactivation of x_1. (B) Outputs of this network in response to X signaling (that is, X input) and Y signaling. Signal x_0 does not lead to the production of y_2, because none of the components of X can activate or inhibit components of Y. Thus, the specificity of X with respect to Y and fidelity of Y with respect to X are complete (see text for definitions). In contrast, y_1 (which might be a kinase of pathway Y) weakly activates x_2 (which might be a transcription factor for pathway X), at a rate characterized by the "leak constant", h_{leak}. Thus, the specificity of Y and the fidelity of X are finite functions of h_{leak} and other key parameters of the network. In particular, in the text it is shown that network specificity is proportional to $1/h_{\text{leak}}$. (C) Depiction of the ratios equal to the specificity of pathway Y and the fidelity of pathway X.

If the two pathways are not interconnected in any way, then signal x_0 will result in the production of x_f, not y_f; likewise, the activation of pathway Y will neither activate nor inhibit pathway X. Thus, the two pathways, and the network they comprise, will exhibit complete specificity. As discussed above, however, interconnections between pathways often exist; in the network in Fig. 2 there is a connection from y_1 to x_2. In many cases, such interconnections serve a purpose: if it is advantageous for a cell to always have pathway X active whenever pathway Y is active (e.g., because the response evoked by X augments or complements the response evoked by Y), then natural selection may have resulted in a network wired such that Y activates X. On the other hand, cross talk between pathways can be undesirable

if it is disadvantageous for pathway Y to influence pathway X (for example, if the response evoked by X is antagonistic or irrelevant to the response evoked by Y).

When pathway Y receives a signal (and X does not), the magnitude of the response of pathway X (if any) provides a measure of the amount of signal crossover. If this crossover represents undesirable leaking, then it should presumably be small compared to both authentic Y signaling (Y output when Y receives a signal) and authentic X signaling (X output when X receives a signal).

Definitions of specificity and fidelity

Previously we defined two properties, specificity and fidelity, that all pathways in a network must possess to avoid paradoxical situations where the input for a given pathway activates another pathway's output more than its own; or where the output for a given pathway is activated more by another pathway's input than by its own (28).

Let us denote the total output of pathway X when the cell is exposed to an input signal x_0 as $X_{\text{out}}|X_{\text{in}}$ (read as "X output given X input", or simply "X given X"). Similarly, let us define the spurious output of pathway Y when the cell is exposed to signal x_0 as $Y_{\text{out}}|X_{\text{in}}$. These quantities should be interpreted as ensemble averages in noisy networks (a detailed analysis is presented further below). The specificity of cascade X (with respect to Y) is the ratio of its authentic output to its spurious output:

$$S_X = \frac{X_{\text{out}}|X_{\text{in}}}{Y_{\text{out}}|X_{\text{in}}} \qquad (1)$$

If pathway X is activated by a given signal and this does not result in any output from pathway Y, the specificity of X with respect to Y in response to that signal is infinite, or complete. However, if there is some cross talk between the pathways, then activation of X will result in some output from Y, and the specificity will be finite. If S_X is <1, the input signal for X promotes the output of pathway Y more than its own output.

Similarly, the specificity of cascade Y is defined as follows:

$$S_Y = \frac{Y_{\text{out}}|Y_{\text{in}}}{X_{\text{out}}|Y_{\text{in}}} \qquad (2)$$

The overall specificity of the network can be measured by the product

$$S_{\text{network}} = S_X S_Y. \qquad (3)$$

We say that a pathway or network "has specificity (of degree k)" if $S \geq k$ for some $k > 1$. Mutual specificity (of degree k) is when both pathways in a network have specificity (of degree k) with respect to each other. The maximum degree of mutual specificity that a network can possibly possess is given by the relationship $k \leq \sqrt{S_{\text{network}}}$.

We define the fidelity of X with respect to Y as the total output of X when X receives a signal (and Y does not) divided by the total output of X when Y receives a signal (and X does not). That is, the fidelity of a pathway is its output when given an authentic signal divided by its output in response to a spurious signal.

$$F_X = \frac{X_{out}|X_{in}}{X_{out}|Y_{in}}, \quad F_Y = \frac{Y_{out}|Y_{in}}{Y_{out}|X_{in}}. \quad (4)$$

A pathway that exhibits fidelity (i.e., $F > 1$) is activated more by its authentic signal than by others. In contrast, if a pathway has fidelity of <1, it is activated more by another pathway's signal than it is by its own. One obvious way for fidelity to be compromised is if a receptor binds promiscuously to several different hormones. However, lack of fidelity may also arise as a consequence of cross talk, as shall be shown further below. As with specificity, the fidelity of the network is the product of the pathway fidelities. Fidelity of degree k and mutual fidelity are also defined similarly to the corresponding expressions for specificity. Note that $S_{network} = F_{network}$, so we will use the term network specificity to mean "network specificity and network fidelity".

We have found specificity, as defined above, to be a useful analytical concept. However, when considering real biological endpoints, specificity, which compares X_{out} to Y_{out}, is essentially comparing apples to oranges. For this reason, fidelity, which compares apples to apples, is perhaps a superior metric when applied to experimental data. More detail concerning how specificity and fidelity can be experimentally measured is presented in the section "Inclusion of background signal levels and experimental measurements" further below.

Alternative definitions of specificity and fidelity that may be useful in some applications would take the form

$$F_X = \frac{X_{out}|X_{in}}{(X_{out}|X_{in} + X_{out}|Y_{in})}, \text{ etc.}$$

With this definition, pathway specificities and fidelities would vary in the interval between 0 and 1, inclusive; thus complete fidelity would be characterized by $F = 1$ rather than F equal to infinity, and F below 0.5 would indicate poor fidelity. As another alternative, Schaber et al. (29) defined a measure they called cross talk (C), which is the reciprocal of our fidelity. This metric varies between 0 and infinity, with 0 indicating complete fidelity (no cross talk) and values above 1 indicating poor fidelity. Herein we use the definitions given in Eqs. 1–4.

A network with aberrant cross talk

As an example of how specificity and fidelity can be calculated in a network of defined architecture, we first consider the simple network shown in Fig. 2. In this network, pathway Y leaks into pathway X, because kinase y_1 is somewhat lacking in substrate selectivity: in addition to phosphorylating its correct target y_2 at a rate proportional to b_2, it also phosphorylates the incorrect target x_2 at rate proportional to h_{leak}.

Let us denote by $\bar{x}_n|X = \int_0^\infty x_n(t)\, dt|_{x_0>0,y_0=0}$ the total amount of product x_n when the cell is exposed to signal x_0 but not to signal y_0. Similarly, $\bar{y}_n|X$ denotes the total amount of y_n under the action of signal x_0. Let x_f and y_f denote the final products of pathways X and Y, respectively, so that $\bar{x}_f|X$ is another way of writing $X_{out}|X_{in}$. For the purposes of the following exposition, we presume that the production of x_f in response to signal y_0 is undesirable.

Our approach, similar to that of Heinrich et al. (30), is to model the enzymatic reactions of signaling pathways using equations that are simplifications of the standard mass action or Michaelis-Menten formulations. These simplifications are made so that exact analytical solutions of the equations can be obtained in most cases. In particular, we assume that the pathways are weakly activated, meaning that the level of component activation is low compared to the total amount of that component in the cell. (In the Supplementary Material, we demonstrate that some of the key results hold even when pathways are strongly activated.) The assumption of weak activation allows signaling cascades to be modeled as a linear system (30,31). For instance, when pathway X is on (and Y is off), the dynamics of signaling in pathway X can be expressed as a simple linear system of ordinary differential equations (ODEs):

$$\dot{x}_1 = a_1 x_0(t) - d_1^x x_1 \quad (5)$$

$$\dot{x}_2 = a_2 x_1 - d_2^x x_2. \quad (6)$$

Here, $x_0(t)$ is the signal function, and x_1 and x_2 are concentrations of the active species of these components at a given moment of time. The parameters a_1 and a_2 are activation rate constants; a_2 is proportional to the rate at which kinase x_1 activates (phosphorylates) target x_2. Similarly, d_1^x and d_2^x are deactivation (or decay) rate constants, and can be thought of as representing phosphatase activity or protein degradation, for example. The term $\dot{x}_1$ is a shorthand notation for (dx_1/dt), the rate of change of component x_1 at a particular moment in time. This is equal to the amount of x_1 being created minus the amount being destroyed at that time. The former is equal to the magnitude of signal x_0 multiplied by the rate constant a_1; the latter is equal to the concentration of x_1 multiplied by the decay rate constant d_1^x. Equations 5 and 6 can be interpreted as equations for average values of the variables, and can be rigorously derived from a stochastic process; this is done later in the article.

The solution of Eqs. 5 and 6 is obtained by integrating both sides from zero to infinity; resulting in the left-hand side of each equation being replaced by zero and x_1 and x_2 being replaced by $\bar{x}_1$ and $\bar{x}_2$, respectively. Rearrangement then yields

$$\bar{x}_1|X = \frac{\bar{x}_0 a_1}{d_1^x} \quad \text{and} \quad \bar{x}_2|X = (\bar{x}_1|X)\frac{a_2}{d_2^x} = \frac{\bar{x}_0 a_1 a_2}{d_1^x d_2^x}. \quad (7)$$

Let us hereafter refer to the level of signal flux to the intermediate component (i.e., x_1 or y_1 in the examples herein) as the signal strength; this will be a product of the magnitude of the input signal and the rate coefficient(s) for the upstream step(s), or $\bar{x}_0 a_1$ in the above example.

The dynamics of pathway Y signaling under the action of y_0 can be similarly expressed as

$$\dot{y}_1 = b_1 y_0(t) - d_1^y y_1 \quad (8)$$
$$\dot{y}_2 = b_2 y_1 - d_2^y y_2. \quad (9)$$

From these it can be determined that

$$\bar{y}_1|Y = \frac{\bar{y}_0 b_1}{d_1^y} \quad \text{and} \quad \bar{y}_2|Y = \frac{\bar{y}_0 b_1 b_2}{d_1^y d_2^y}. \quad (10)$$

Furthermore, it is obvious by inspection that $\bar{y}_2|X = 0$. Thus, the expressions for S_X and F_Y have zero in the denominator, so X can be considered to have infinite, or complete, specificity (with respect to Y), and Y can be considered to have complete fidelity. The calculation of $\bar{x}_2|Y$ requires modifying Eq. 6 so that it becomes (Eq. 6a):

$$\dot{x}_2 = a_2 x_1 + h_{leak} y_1 - d_2^x x_2. \quad$$

Note that the first term of Eq. 6a is zero when pathway X is off and the second term is zero when Y is off. From Eqs. 6a and 8 it can be determined that

$$\bar{x}_2|Y = \frac{\bar{y}_0 b_1 h_{leak}}{d_1^y d_2^x}. \quad (11)$$

Thus,

$$S_Y = \frac{b_2 d_2^x}{h_{leak} d_2^y}, \quad F_X = \frac{\bar{x}_0 a_1 a_2 d_1^y}{\bar{y}_0 b_1 h_{leak} d_1^x}. \quad (12)$$

Hence, both quantities are decreasing functions of the ''leakage rate'' h_{leak}, and will be very large if h_{leak} is very small.

Network specificity is undefined when one pathway has complete specificity; only if there is bidirectional crossover does it make sense to calculate network specificity. To add crossover in the other direction, assume that kinase x_1 also lacks complete selectivity, and phosphorylates substrate $y2$ at rate j_{leak}. This results in the architecture shown in Fig. 3. In this case, Eq. 12 still holds for S_Y and F_X, and in addition

$$S_X = \frac{a_2 d_2^y}{j_{leak} d_2^x}, \quad F_Y = \frac{\bar{y}_0 b_1 b_2 d_1^x}{\bar{x}_0 a_1 j_{leak} d_1^y}, \quad S_{network} = \frac{a_2}{j_{leak}} \frac{b_2}{h_{leak}}. \quad (13)$$

The ratios a_2/j_{leak} and b_2/h_{leak} can be taken as measures of the selectivity of kinases x_1 and y_1, respectively, for their correct versus incorrect targets. As can be seen, the specificity of the network in this case is equal to the product of these selectivities. Moreover, both mutual specificity and mutual fidelity are rather easily achieved. For instance, in the case of symmetric network parameters ($\bar{x}_0 = \bar{y}_0, a_1 = b_1, d_1^x = d_1^y$, etc.), the conditions for both are $a_2/j_{leak} > 1$, $b_2/h_{leak} > 1$. (To be precise, $a_2/j_{leak} \geq k$; $b_2/h_{leak} \geq k$; $k > 1$.)

Fig. 3 shows some example solutions of this network under the action of signal x_0 (''X_{in}'') and signal y_0 (''Y_{in}''), and the resulting specificity and fidelity values. The network in Fig. 3 A possesses both mutual specificity and mutual fidelity. As can be seen, however, one can envisage a network with
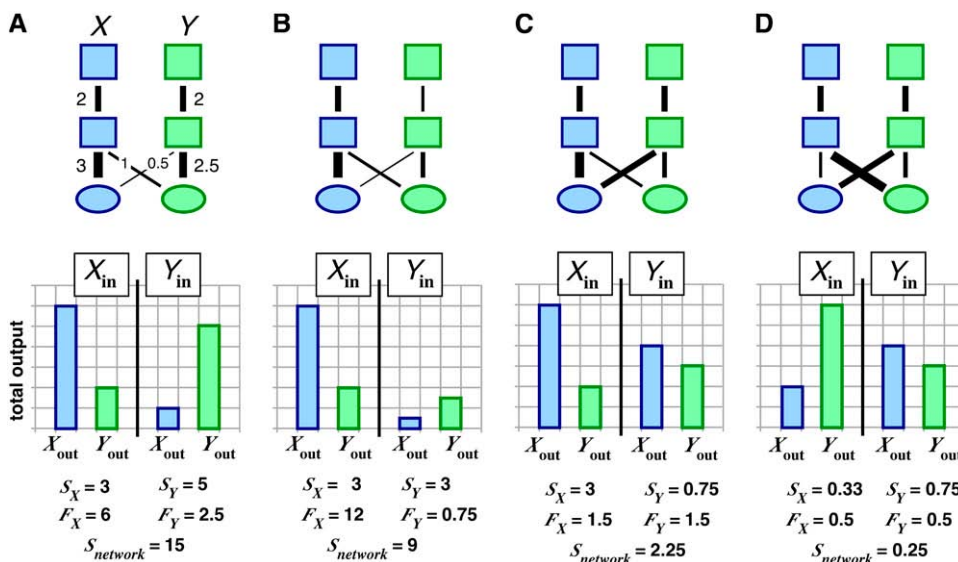

FIGURE 3 The output of four signaling networks exhibiting different degrees of specificity and fidelity. The bars on the graph represent the total output of signaling pathways X and Y (X_{out} and Y_{out}, respectively) under the condition where pathway X is receiving a signal and pathway Y is not (X_{in}), and visa versa (Y_{in}). The results correspond to solutions of the network described in Eqs. 5–13. All parameter values were set equal to 1 except as specified below. The thickness of the links connecting the component nodes is proportional to the rate coefficient for that reaction (see Fig. 2 A; j is the coefficient for the connection from x_1 to y_2). (A) This network exhibits both mutual specificity and mutual fidelity. Networks such as this are presumably the most useful to the organism. Rate coefficients are $a_1 = 2, a_2 = 3, b_1 = 2, b_2 = 2.5, h = 0.5, j = 1$. (B) This network displays mutual specificity but not mutual fidelity. Pathway Y does not exhibit fidelity with respect to pathway X, because Y output when Y is on is less than Y output when X is on. Parameter values are $a_1 = 2, a_2 = 3, b_1 = 1, b_2 = 1.5, h = 0.5, j = 1$. (C) This network possesses mutual fidelity, but pathway Y does not exhibit specificity with respect to X. Parameter values are $a_1 = 2, a_2 = 3, b_1 = 2, b_2 = 1.5, h = 2, j = 1$. (D) This network possesses neither mutual specificity nor mutual fidelity. Parameter values are $a_1 = 2, a_2 = 1, b_1 = 2, b_2 = 1.5, h = 2, j = 3$.

mutual specificity but without mutual fidelity (Fig. 3 B), and visa versa (Fig. 3 C). Furthermore, a given pathway can exhibit both specificity and fidelity, or only one or the other, or neither (Fig. 3 D).

Cascades that share components

For the remainder of this article, we will examine the situation where two signaling pathways share one or more common elements (see Fig. 4 A). Without any further assumptions, this class of networks can be represented by the simple architecture shown in Fig. 4 A, and it can be expressed as the following ODEs:

$$\dot{x}_1 = a_1 x_0(t) + b_1 y_0(t) - d_1 x_1 \quad (14)$$

$$\dot{x}_2 = a_2 x_1 - d_2^x x_2 \quad (15)$$

$$\dot{y}_2 = b_2 x_1 - d_2^y y_2. \quad (16)$$

We refer to this as the ''basic architecture'', because the network lacks any enhancements designed to promote specificity. In the cases we will consider, we assume the network only receives one of the two signals at a time. Thus, if $x_0(t)$ is positive for some duration of time, then $y_0(t)$ is identically zero, and visa versa; either the first or second term of Eq. 14 is equal to zero, depending upon which of the two signals the network is receiving. Assume that signals $x_0(t)$ and $y_0(t)$ have the duration t_x and t_y, respectively, and average magnitudes equal to the constants $\hat{x}_0$ and $\hat{y}_0$. Then, when X is ''on'' and Y is ''off'',

$$\bar{x}_1|X = \frac{\hat{x}_0 t_x a_1}{d_1}, \quad \bar{x}_2|X = \frac{\hat{x}_0 t_x a_1 a_2}{d_1 d_2^x}, \quad \bar{y}_2|X = \frac{\hat{x}_0 t_x a_1 b_2}{d_1 d_2^y}. \quad (17)$$

Furthermore, when pathway Y is ''on'' and X is ''off'' (that is, $y_0(t) > 0$ and $x_0(t) = 0$),

$$\bar{x}_1|Y = \frac{\hat{y}_0 t_y b_1}{d_1}, \quad \bar{x}_2|Y = \frac{\hat{y}_0 t_y b_1 a_2}{d_1 d_2^x}, \quad \bar{y}_2|Y = \frac{\hat{y}_0 t_y b_1 b_2}{d_1 d_2^y}. \quad (18)$$

From these expressions we can calculate that

$$S_X = \frac{a_2 d_2^y}{b_2 d_2^x}, \quad S_Y = \frac{b_2 d_2^x}{a_2 d_2^y}, \quad F_X = \frac{\hat{x}_0 t_x a_1}{\hat{y}_0 t_y b_1}, \quad F_Y = \frac{\hat{y}_0 t_y b_1}{\hat{x}_0 t_x a_1}. \quad (19)$$

These quantities are quite easy to understand intuitively. The expressions for fidelity are simply ratios of signal strength multiplied by signal duration, that is, ratios of the total amount of signal flowing into the shared component. In contrast, the quantities for specificity report on signaling downstream of the activation of the shared component. In the case of S_X, the numerator contains a coefficient (a_2) that positively influences X_{out} and a coefficient (d_2^y) that negatively influences Y_{out}, whereas the coefficients in the denominator act conversely.

Note from Eq. 19 that S_X is the reciprocal of S_Y, and F_X is the reciprocal of F_Y. Thus, because $S_{network} = S_X S_Y = F_X F_Y$, $S_{network} = 1$. Thus, the basic architecture does not exhibit overall network specificity, nor does it exhibit mutual specificity or mutual fidelity (28).

Insulating mechanisms: combinatorial signaling

Real cellular signaling networks that share components contain one or more insulating mechanisms that are thought to contribute to specificity and fidelity, some of which are shown in Fig. 4. First, in combinatorial signaling, the simultaneous
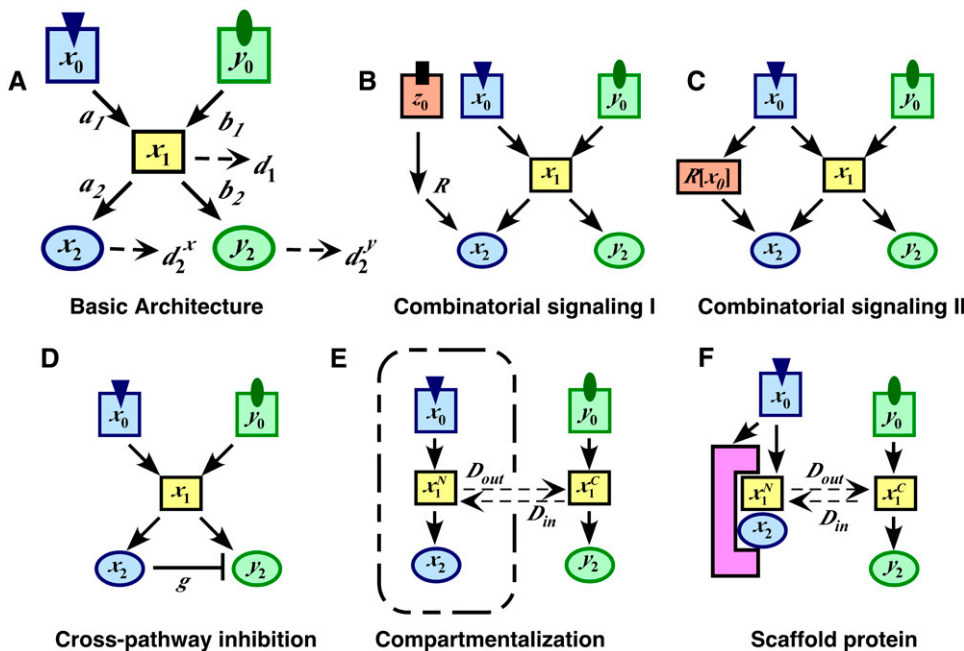
FIGURE 4 Signaling network with shared components. (A) The simplest such network, herein referred to as the ''basic architecture'', shown with activation and decay rate constants. Component x_1 is common to pathways X and Y. This network cannot achieve specificity and fidelity. (B–F) Elaborations to the basic architecture that are found in nature and have been proposed to promote specificity. (B) Combinatorial signaling with an independent, parallel input provided by pathway Z. (C) Combinatorial signaling via the branching and reintegration of pathway X. (D) Cross-pathway inhibition. (E) Compartmentalization. (F) The action of a scaffold protein.

action of two or more different signals may be required to evoke a response, so that the output of a pathway is determined by the combination of signals acting on a network (Fig. 4 *B*). For example, the survival of epithelial cells requires two signals, one provided by growth factors and transmitted by the MAPK pathway, and one provided by cell attachment (32), and Wnt and BMP signals combine to determine whether neural crest stem cells will differentiate (33). Another type of combinatorial signaling occurs when a pathway branches into two subpathways (one that contains shared components and one that doesn't) that are reintegrated at a point further downstream, so that the response to a given signal is determined by the combination of subpathways activated by that signal (Fig. 4 *C*) (34). Combinatorial signaling requires that a downstream component (such as x_2 in Fig. 4, *B* and *C*) is able to act as a molecular ''AND gate'' or ''coincidence detector'' that integrates two separate inputs (35,36). One of these inputs may be a component that is shared with another pathway (such as kinase x_1, which is shared with pathway Y). If the other input is not shared with Y, then this may be exploited to enhance the specificity of the XY network. Some examples of proteins that function as signal integrators include Smad1, which integrates MAPK and TGFβ signals (37), and the estrogen receptor, which integrates MAPK and estrogen hormone signals (38). In addition, DNA regulatory elements such as the *Drosophila eve-skipped* enhancer can also act as signal integrators (39).

Indeed, combinatorial signaling is used in the yeast mating and invasive growth signaling network to regulate a subset of filamentation genes during invasive growth. The first signal is relayed via the cell surface protein Msb2, which senses localized turgor pressure (or some other mechanical force, presumably) and sends a signal via the MAPK cascade to activate the Tec1 transcription factor (40). A second, glucose limitation signal is sensed by a Snf1 protein kinase, which inhibits a transcriptional repressor known as Nrg (41). Both Tec1 and Nrg bind to the promoters of certain genes required for invasive growth, and efficient activation of these genes requires both Tec1 activation and removal of Nrg-dependent repression (15). Thus, optimal haploid invasive growth requires both a mechanical signal and a glucose limitation signal.

To add combinatorial signaling for pathway X, we modify Eq. 15 of the basic architecture characterized above (Eqs. 14–16) by adding a single term $R[x_0]$, so that (Eq. 15a):

$$\dot{x}_2 = a_2 R[x_0] x_1 - d_2^x x_2.$$

Here, $R[x_0]$ represents the combinatorial input. Thus, target x_2 is the signal integrator, or ''AND gate'': x_2 activity depends on two separate inputs, R and x_1. If either input is zero then x_2 is also zero. Note the case when the coefficient $R[x_0] \equiv 1$ is identical to the basic architecture. To add the influence of an independent, parallel pathway Z, as in Fig. 4 *B*, we set

$$R[x_0] = \begin{cases} 1, & X\,is\,on\,(i.e., x_0(t) > 0) \\ k_{\text{leak}}, & X\,is\,off\,(i.e., x_0(t) = 0), 0 \le k_{\text{leak}} \le 1 \end{cases}. \quad (20)$$

Here we assume that Z is activated concurrently with X, because the cell is usually exposed to both signals at the same time. Further, we assume that Z is mostly, but not completely, off when X is off. The basal activity of Z when X is off is characterized by the leakage rate k_{leak}, and provides a ''back door'' via which Y can leak into X.

Alternatively, if the situation shown in Fig. 4 *C* applies, where X branches and is reintegrated at x_2, then the flux through the X-dedicated subpathway will be proportional to the signal x_0. This can be represented by setting $R[x_0] = x_0(t) + k_{\text{leak}}$. Here the leakage constant k_{leak} represents the basal activity of the X-dedicated subpathway, and the requirement that $k_{\text{leak}} \le 1$ can be dropped. Because the activation of the shared kinase x_1 is also proportional to signal x_0, branching and reintegration leads to ''signal multiplication'' with itself, a phenomenon that has been examined for a single pathway by Heinrich et al. (30).

Suppose for simplicity that signal $x_0(t)$ is a square pulse of amplitude $\hat{x}_0$ and length t_x, and $y_0(t)$ is a square pulse of amplitude $\hat{y}_0$ and length t_y. Specificity for all three models (Fig. 4, *A–C*) are then given by

$$S_X = \frac{a_2 d_2^y}{b_2 d_2^x}(R[\hat{x}_0] - \xi), \quad S_Y = \frac{b_2 d_2^x}{a_2 d_2^y}\frac{1}{R[0]}, \quad S_{\text{network}} = \frac{R[\hat{x}_0] - \xi}{R[0]}. \quad (21)$$

Here $\xi = (1 - e^{-d_1 t_x})(R[\hat{x}_0] - R[0])/d_1 t_x$ is a positive quantity that becomes insignificant if the duration of the signal x_0 exceeds $1/d_1$, the characteristic time for the deactivation of kinase x_1. We can see that for shorter signals, specificity is always lower, and it reaches a saturation level for relatively long x_0; this level is given by

$$S_{\text{network}} = \frac{R[\hat{x}_0]}{R[0]} =$$

$$\begin{cases} 1 & \text{for the basic architecture,} \\ 1/k_{\text{leak}}, & \text{with an independent, parallel input,} \\ (\hat{x}_0 + k_{\text{leak}})/k_{\text{leak}}, & \text{under branching/reintegration.} \end{cases} \quad (22)$$

In both types of combinatorial signaling, specificity is inversely proportional to the amount of leakage, and by making k_{leak} small, it is possible to obtain arbitrarily high levels of network specificity.

For all three models, the expressions for fidelity are:

$$F_x = \frac{\hat{x}_0 t_x a_1}{\hat{y}_0 t_y b_1}\frac{(R[\hat{x}_0] - \xi)}{R[0]}, \quad F_y = \frac{\hat{y}_0 t_y b_1}{\hat{x}_0 t_x a_1}. \quad (23)$$

In summary, compared to the basic architecture, combinatorial signaling raises F_X, S_Y, and S_{network}, and has no effect on F_Y. Under the branching/reintegration scheme, S_X is also increased.

Interestingly, with combinatorial signaling there are obstacles to obtaining mutual specificity and fidelity that place additional requirements on the characteristics of the network. In the case of an independent parallel input, achieving mutual specificity of degree k requires both $k_{\text{leak}} \leq 1/k^2$ and $\alpha \geq k$, where $\alpha = a_2 d_2^y / b_2 d_2^x = (a_2/d_2^x)/(b_2/d_2^y)$. Clearly, the second condition is impossible in the case of symmetric network parameters. Note that (a_2/d_2^x) is the ''local sensitivity coefficient'' for x_2 production—the percent of change in x_2 caused by a 1% change in x_1 at steady state (42). Similarly, (b_2/d_2^y) is the local sensitivity coefficient for y_2 generation; thus α measures relative signal transfer from x_1 to x_2 vs. y_2. To obtain mutual specificity of a reasonably high degree, this must be correspondingly high. One way to achieve this is to reduce b_2, the rate constant for the phosphorylation of target y_2 by kinase x_1, or in other words, to make a y_2 a poor substrate for x_1. This analysis suggests that maximizing the performance objective of mutual specificity may favor a seemingly paradoxical situation where an authentic substrate of a kinase is a poor target for that kinase. Mutual fidelity also cannot be obtained with symmetric network parameters and signal inputs. Achieving mutual fidelity requires greater strength or/and duration of the Y signal compared to the X signal, and low value of k_{leak}. The branching/reintegration scheme (Fig. 4 C) makes it easier to achieve mutual specificity and mutual fidelity, but the latter still requires a stronger or longer Y signal feeding into kinase x_1.

Fig. 5 shows a typical design of an optimized network featuring combinatorial signaling. The figure illustrates how achieving the goal of specificity shapes the network design so that the rate coefficients leading from y_0 to x_1 and from x_1 to x_2 are large, whereas those from x_0 to x_1 and from x_1 to y_2 are small.

The obstacles to obtaining mutual specificity and fidelity in the above model arise because an insulating mechanism has been added to just one of the two pathways in the network. If a combinatorial input was also provided to pathway Y, then it would be straightforward to achieve both mutual specificity and fidelity, even in the case of symmetric network parameters, provided only that the leakage constants were small.

Cross-pathway inhibition

Cross-pathway inhibition (Fig. 4 D) occurs when a downstream component of pathway X inhibits a downstream component of pathway Y. An example of this type of inhibition has recently been discovered in yeast, where it was shown that Fus3, the MAP kinase in the mating pathway, phosphorylates Tec1, a transcriptional regulator for invasive growth, and thereby accelerates the degradation of Tec1 (21,22,43,44). This promotes specificity during mating by preventing Kss1$^{\text{MAPK}}$, which is also activated by mating pheromone, from activating Tec1-dependent transcription of filamentation genes (Fig. 6). This situation can be represented by the general architecture shown in Fig. 4 D, where component x_2 inhibits y_2. To model inhibition, we assume that the effective decay rate of the inhibited component is a growing function of the concentration of the inhibiting component. This results in a modification of Eq. 16 (Eq. 16a):

$$\dot{y}_2 = b_2 x_1 - (d_2^y + g x_2) y_2.$$

Here, g is a rate coefficient that relates the amount of x_2 to the increased decay of y_2. Clearly, the mechanism of inhibition is nonlinear by nature. However, it is possible to solve the system of equations (Eqs. 14, 15, and 16a) under the assumption that the signals x_0 and y_0 are square pulses of amplitudes $\hat{x}_0$ and $\hat{y}_0$ and lengths t_x and t_y, which are long compared to the half-lives of the activated proteins, $1/d_{1,2}^{x,y}$ (i.e., the steady-state approach). This approach yields

$$S_X = \frac{a_2 d_2^y}{b_2 d_2^x}\left(1 + \frac{\hat{x}_0 a_1 a_2 g}{d_1 d_2^x d_2^y}\right),$$

$$S_Y = \frac{b_2 d_2^x}{a_2 d_2^y}\left(1 + \frac{\hat{y}_0 b_1 a_2 g}{d_1 d_2^x d_2^y}\right)^{-1},$$

$$S_{\text{network}} = \left(\frac{d_1 d_2^x d_2^y + \hat{x}_0 a_1 a_2 g}{d_1 d_2^x d_2^y + \hat{y}_0 b_1 a_2 g}\right). \tag{24}$$

$S_X = 3.16 \qquad S_Y = 3.16 \qquad F_X = 3.16 \qquad F_Y = 3.16 \qquad S_{network} = 10$
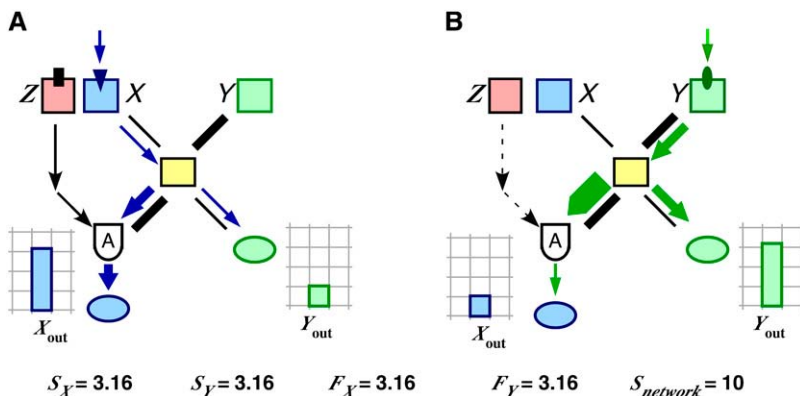
FIGURE 5 Representative network featuring the ''combinatorial signaling'' insulating mechanism with an independent, parallel input, as shown in Fig. 4 B. The abstract action of the molecular AND gate is represented by the symbol inscribed with an ''A''. The thickness of the lines connecting the components represents the magnitudes of the rate coefficients. Network parameters are $k_{\text{leak}} = 0.1$, $x_0 = 1, y_0 = 1, t_X = 1, t_y = 1$. Rate coefficients are $a_1 = b_2 = 1$, $b_1 = a_2 = \sqrt{1/k_{\text{leak}}} = 3.16$. Deactivation rates are not shown and were set equal to 1. The thickness of the arrows leading from one component to another represent the signal flux through that point of the network under the action of signal x_0 (A) or y_0 (B). The design shown optimizes network specificity, mutual specificity, and mutual fidelity, given parameters a_1, b_1, a_2, b_2 chosen from the range $\{0.5–4\}$.
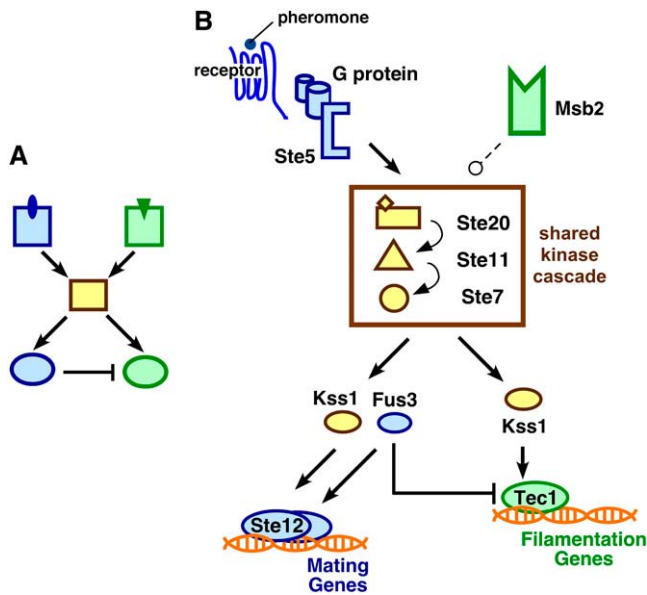
FIGURE 6 The ''cross-pathway inhibition'' insulating mechanism. (*A*) The simplest abstract network featuring cross-pathway inhibition. (*B*) A real network containing cross-pathway inhibition, the yeast mating/invasive growth signaling network.

Thus, compared to the basic architecture (see Eq. 19), cross-pathway inhibition increases S_X and decreases S_Y. The specificity of the network will be >1 only if $\hat{x}_0 a_1 > \hat{y}_0 b_1$; that is, the signal strength for pathway X must be stronger than that for pathway Y.

The condition for mutual specificity of degree k is equivalent to two simultaneous inequalities,

$$\alpha(1 + \hat{x}_0 a_1 Q) > k, \quad \frac{1}{\alpha(1 + \hat{y}_0 b_1 Q)} > k,$$

where we previously defined $\alpha = (a_2/d_2^x)/(b_2/d_2^y)$, and $Q = a_2 g/(d_1 d_2^x d_2^y)$. Two necessary (but not sufficient) conditions to achieve this are $\hat{x}_0 a_1 > \hat{y}_0 b_1$ and $\alpha < 1/k$. The condition $\hat{x}_0 a_1 > \hat{y}_0 b_1$ requires strong relative signaling from signal x_0 to x_1. Moreover, since α, a ratio of sensitivity coefficients, measures the efficiency of signal transmission from x_1 to x_2 vs. y_2, the condition $\alpha < 1/k$ requires weak relative signaling from x_1 to x_2. Thus, this scheme places significant constraints on the allowable signal flux through different steps of pathway X, and would seem to require significant signal dampening down the pathway.

The fidelity values of the two cascades are given by

$$F_X = \frac{\hat{x}_0 t_x a_1}{\hat{y}_0 t_y b_1}, \quad F_Y = \frac{\hat{y}_0 t_y b_1}{\hat{x}_0 t_x a_1}\left(\frac{d_1 d_2^x d_2^y + \hat{x}_0 a_1 a_2 g}{d_1 d_2^x d_2^y + \hat{y}_0 b_1 a_2 g}\right). \quad (25)$$

Thus, F_X is the same as in the basic architecture and F_Y will be greater than in the basic architecture only if $\hat{x}_0 a_1 > \hat{y}_0 b_1$. Mutual fidelity is even more difficult to achieve than mutual specificity. However, it is possible to achieve mutual fidelity to some degree by increasing the strength of signal x_0

compared to the strength of signal y_0 while keeping signal y_0 sufficiently long compared to signal x_0. In the ''best'' case, where the decay rates are very small compared to the other constants, mutual fidelity of degree k could be obtained providing $\hat{x}_0 a_1/\hat{y}_0 b_1 > k$ and $t_y/t_x > k^2$. Hence, the requirement of mutual fidelity imposes conditions on both the relative strength and duration of the input signals. Mutual fidelity is impossible for signals of equal length or equal strength.

Fig. 7 depicts a representative network featuring cross-pathway inhibition of Y by X as its only insulating mechanisms. The network achieves some degree of mutual specificity but cannot achieve mutual fidelity, because the signal durations are similar. Obtaining mutual specificity constrains the network design so that the rate coefficients leading from x_0 to x_1 and from x_1 to y_2 are large, whereas those from y_0 to x_1 and from x_1 to x_2 are small.

Compartmentalization and the sequestering function of scaffold proteins

Elsewhere we analyzed the insulating mechanism of compartmentalization and compared it to the sequestering function of scaffold proteins (28). In compartmentalization, different pathways are localized to different compartments or regions of the cell, such as the nucleus and the cytosol. Leaking between pathways can only occur if a shared component, while active, moves from one compartment to another (Fig. 4 *E*).

Signaling scaffolds are proteins that bind to two or more consecutively acting components of a pathway and accelerate the rate of reaction between them (Fig. 4 *F*). For example,

$S_X = 2.25 \quad S_Y = 2.0 \quad F_X = 8.0 \quad F_Y = 0.56 \quad S_{network} = 4.5$
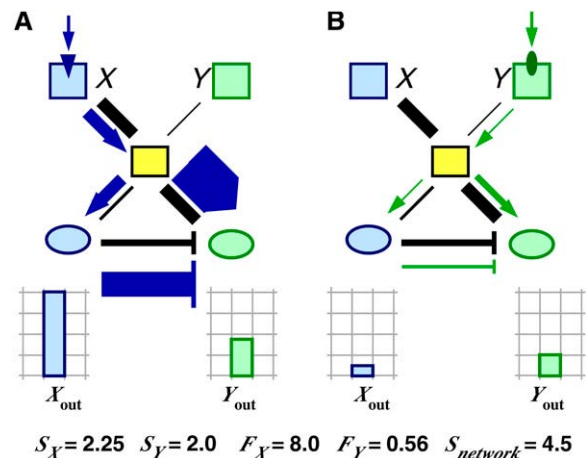
FIGURE 7 Representative network featuring cross-pathway inhibition as its only insulating mechanism. The thickness of the lines connecting the components represents the magnitudes of the rate coefficients. Signal magnitudes and durations are $x_0 = 1$, $y_0 = 1$, $t_X = 1$, $t_y = 1$. Rate coefficients are $a_1 = 4$, $a_2 = 1$, $b_1 = 0.5$, $b_2 = 4$, $g = 2$. Deactivation rates are not shown and were set equal to 1. The thickness of the arrows leading from one component to another represent the signal flux through that point of the network under the action of signal x_0 (*A*) or y_0 (*B*). The design shown optimizes mutual specificity given parameters a_1, b_1, a_2, b_2, g chosen from the set $\{0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4\}$.

yeast Ste5 protein is a scaffold protein in the mating pathway. It binds to the G-protein that is an upstream component of the mating pathway, to the shared intermediate kinases Ste11$^{\text{MAP3K}}$ and Ste7$^{\text{MAP2K}}$, and to the mating-specific downstream kinase Fus3$^{\text{MAPK}}$ (45). As such, it enables a pathway-specific upstream component to activate the shared kinases, and then helps the shared kinases to activate a pathway-specific downstream kinase (Fig. 8 A). This type of mechanism may enhance specificity if the movement of the active shared kinases on and off the scaffold is limited. In particular, if the reactions between the kinases can only happen while bound to the scaffold (or are much more efficient on scaffold than off scaffold), then this sequestering action of the scaffold is formally equivalent to compartmentalization, and both mechanisms can be represented by the same set of equations, as follows:

$$\dot{x}_1^{\text{N}} = a_1 x_0(t) - D_{\text{out}} x_1^{\text{N}} + D_{\text{in}} x_1^{\text{C}} - d_1^{\text{x}} x_1^{\text{N}} \qquad (26)$$

$$\dot{x}_1^{\text{C}} = b_1 y_0(t) - D_{\text{in}} x_1^{\text{C}} + D_{\text{out}} x_1^{\text{N}} - d_1^{\text{y}} x_1^{\text{C}} \qquad (27)$$

$$\dot{x}_2 = a_2 x_1^{\text{N}} - d_2^{\text{x}} x_2 \qquad (28)$$

$$\dot{y}_2 = b_2 x_1^{\text{C}} - d_2^{\text{y}} y_2. \qquad (29)$$

Here, x_1^{N} is the concentration of active kinase x_1 in the **N**ucleus (or a**N**chored, or o**N**, the scaffold) and x_1^{C} is the concentration of active kinase x_1 in the **C**ytosol; D_{out} is the rate constant at which x_1 exits the nucleus/scaffold and enters the cytosol, and D_{in} is the rate at which x_1 in the cytosol enters the nucleus or binds to the scaffold. The parameters d_1^{x} and
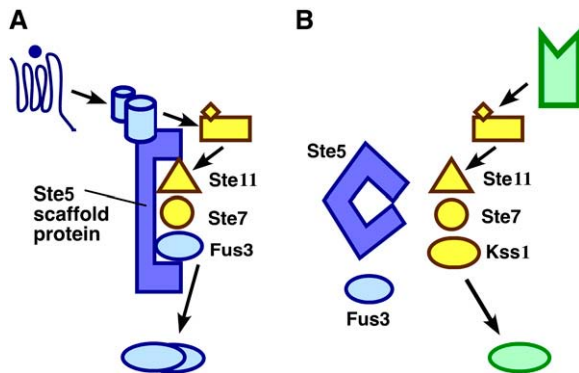

FIGURE 8 Action of a selectively activated scaffold. The cartoon is based upon a model recently proposed for the action of yeast Ste5 (23). (A) During mating, Ste5 is activated by the mating-pathway-specific G-protein. Activated Ste5 is competent to act as a scaffold by binding to the shared kinases Ste11$^{\text{MAP3K}}$ and Ste7$^{\text{MAP2K}}$ and promoting the Ste7-dependent activation of the mating-pathway-specific kinase Fus3$^{\text{MAPK}}$. (B) When cells are not mating, Ste5 is in an inactive conformation and cannot channel signals from the shared kinases to Fus3. Thus, even if the cell is undergoing invasive growth or experiencing other stimuli that activate some of the shared kinases, the mating response is not activated. In these situations, however, it is likely that some minor fraction of the Ste5 in the cell is in the active conformation; in the mathematical model this fraction is represented by the parameter k_{leak}.

d_1^{y} are the deactivation constants for x_1 in the nucleus/on the scaffold and in the cytosol, respectively; for example, if x_1 is activated by phosphorylation, d_1^{x} can denote the rate of dephosphorylation of scaffold-bound x_1. Note from Eq. 29 that y_2 only arises via cytosolic x_1, and note from Eq. 27 that, when X is on and Y is off, cytosolic x_1 only arises by the relocation/dissociation of nuclear/scaffold-activated x_1.

The equations for specificity and fidelity are:

$$S_{\text{X}} = \frac{a_2 d_2^{\text{y}}(d_1^{\text{y}} + D_{\text{in}})}{b_2 d_2^{\text{x}} D_{\text{out}}}, \quad S_{\text{Y}} = \frac{b_2 d_2^{\text{x}}(d_1^{\text{x}} + D_{\text{out}})}{a_2 d_2^{\text{y}} D_{\text{in}}}, \qquad (30)$$

$$F_{\text{X}} = \frac{\hat{x}_0 t_{\text{x}} a_1(d_1^{\text{y}} + D_{\text{in}})}{\hat{y}_0 t_{\text{y}} b_1 D_{\text{in}}}, \quad F_{\text{Y}} = \frac{\hat{y}_0 t_{\text{y}} b_1(d_1^{\text{x}} + D_{\text{out}})}{\hat{x}_0 t_{\text{x}} a_1 D_{\text{out}}}, \qquad (31)$$

$$S_{\text{network}} = F_{\text{network}} = \frac{(d_1^{\text{y}} + D_{\text{in}})(d_1^{\text{x}} + D_{\text{out}})}{D_{\text{in}} D_{\text{out}}} > 1. \qquad (32)$$

It can be seen that network specificity is >1, and thus is greater than in the basic architecture. Each of four quantities, S_{X}, S_{Y}, F_{X}, and F_{Y}, differ from the corresponding values in the basic architecture by a factor that is a ratio of an exchange rate constant D plus a decay constant d divided by an exchange rate constant. For example, S_{X} differs by a factor of $(d_1^{\text{y}} + D_{\text{in}})/D_{\text{out}}$ from the basic architecture result. This makes sense: S_{X} is favored by having small D_{out} (so that very little x_1 leaks out of the nucleus/off the scaffold), and large D_{in} and d_1^{y} (so that any x_1 that does leak out is likely to either move back in or decay). In the case of symmetric network parameters, the condition $D_{\text{out}} - d_1^{\text{y}} < D_{\text{in}} < D_{\text{out}} + d_1^{\text{x}}$ guarantees mutual specificity and fidelity of the cascades X and Y. If $D_{\text{in}} = D_{\text{out}} = D$ and $d_1^{\text{x}}, d_1^{\text{y}} > 0$, then this condition is automatically satisfied, and provides mutual specificity and fidelity of degree $k = (d_1 + D)/D$, where d_1 is the lesser of $d_1^{\text{x}}, d_1^{\text{y}}$. Compartmentalization or spatial separation makes "leakage" ineffective as long as the rates of leakage balance each other, and are small compared to the deactivation rates.

Selective activation of scaffold proteins

Another way in which a scaffold might promote specificity is if the scaffold is only in a conformation capable of productively binding the shared kinase(s) during authentic signaling. This mechanism has been termed "selective activation" (23). For example, it has been proposed that the yeast Ste5 scaffold is found in an active conformation only during mating (Fig. 8 A). During invasive growth signaling, Ste5 exists in a "closed" conformation (or is localized in the wrong place or is for some other reason inactive) and so cannot channel signals from the shared kinase Ste7$^{\text{MAP2K}}$ to the mating-specific kinase Fus3$^{\text{MAPK}}$ (Fig. 8 B) (23,46).

To model the selective activation of a scaffold, we modify Eqs. 26 and 27 so that they become (Eqs. 26a and 27a):

$$\dot{x}_1^{\text{N}} = a_1 x_0(t) - D_{\text{out}} x_1^{\text{N}} + R[x_0] D_{\text{in}} x_1^{\text{C}} - d_1^{\text{x}} x_1^{\text{N}}$$

$$\dot{x}_1^{\text{C}} = b_1 y_0(t) - R[x_0] D_{\text{in}} x_1^{\text{C}} + D_{\text{out}} x_1^{\text{N}} - d_1^{\text{y}} x_1^{\text{C}},$$

where $R[x_0]$, as defined previously (Eq. 20), assumes a value of 1 during X signaling and a value k_{leak} between 0 and 1 in the absence of X signaling. Thus, k_{leak} represents the relative basal amount of active scaffold present when X is off; if k_{leak} is 0, there is no scaffold when X is off, and there is no way for Y to leak into X. The resultant values for S_Y and network specificity are then increased by a factor of $1/k_{leak}$ relative to the previous model:

$$S_Y = \frac{b_2 d_2^x (d_1^x + D_{out})}{k_{leak} a_2 d_2^y D_{in}} \tag{33}$$

$$S_{network} = F_{network} = \frac{(d_1^y + D_{in})(d_1^x + D_{out})}{k_{leak} D_{in} D_{out}} > 1. \tag{34}$$

Both F_X and F_Y also increase (provided $k_{leak} < 1$), and only S_X is unchanged by the addition of selective activation. Note that Eqs. 33 and 34 are strictly valid only in the steady state, i.e., if the signal durations t_x, t_y are long compared to the inverse of the eigenvalues of the linear matrix that appears in system (Eqs. 26a and 27a).

In the Supplementary Material, a more elaborate model of scaffolding is presented that is described by a nonlinear system of six differential equations, and yet is still solvable. This model includes the formation of a complex between inactive kinase x_1 and the scaffold and the activation of x_1 on the scaffold, allows both events to be dependent on the signal, and differs in several other details from the above models. Despite these differences, the expressions for network and pathway specificity and fidelity are very similar to those given above. Thus the simpler models appear to capture some of the key features of the specificity-promoting qualities of scaffold proteins.

In summary, scaffold proteins can enhance specificity both by sequestration, a mechanism that resembles compartmentalization, and by selective activation, a mechanism that resembles combinatorial signaling. As such, a scaffold can in principal provide a highly effective insulating mechanism. Indeed, scaffolds are often spatially localized, which would result in an insulating mechanism more effective than either compartmentalization or scaffolding per se. To what extent scaffold proteins use sequestration and/or selective activation to enhance specificity is an area of active experimental investigation. A recent study of the yeast Ste5 protein suggests that selective activation may be more important than sequestration for this scaffold (23). In terms of the model, Ste5 might be considered to be a scaffold for which D_{in} and D_{out} are high relative to d_1^x and d_1^y, and k_{leak} is low.

The effect of noise in signaling networks on specificity and fidelity

Signaling networks are noisy systems, and there has been much work on modeling noise in chemical signaling (see, for example, (47–52)). In this section we examine the effects of noise on specificity and fidelity. We conclude that the effects of noise usually ''average out'' when specificity and fidelity

are calculated, and therefore that the simpler deterministic treatment presented above is generally valid.

There are two sources of noise: internal and external (53). Internal noise is due to the stochastic nature of the collisions and reactions of the proteins involved. External noise includes all sources of noise not directly related to the proteins involved in our description, such as noise from other molecules that affect the system but that are not explicitly described in the model. To model the effect of noise on specificity of signaling networks, we will first derive a chemical Langevin equation (54) to account for the internal noise, and describe how external noise can also be included.

Let us start from a simple linear cascade, $x_0 \xrightarrow{a_1} x_1 \xrightarrow{a_2} x_2$, where species x_1 and x_2 have the decay rates of d_1^x and d_2^x, respectively. Let us denote by i, j, and k the number of molecules of proteins x_0, x_1 and x_2, respectively. We assume the following Poisson process: in an infinitesimal time interval, Δt, the following changes can occur:

$$j \to j + 1 \; with \; probability \; i a_1 \Delta t,$$
$$j \to j - 1 \; with \; probability \; j d_1^x \Delta t,$$
$$k \to k + 1 \; with \; probability \; j a_2 \Delta t,$$
$$k \to k - 1 \; with \; probability \; k d_2^x \Delta t,$$

No change with probability $1 - (ia_1 + ja_2 + jd_1^x + kd_2^x)\Delta t$.

Let us denote by $\varphi_{jk}(t)$ the probability to have j molecules of type x_1 and k molecules of type x_2 at time t. We have the following Kolmogorov (master) equation:

$$\dot{\varphi}_{jk} = \varphi_{j-1,k} i a_1 + \varphi_{j+1,k}(j+1)d_1^x + \varphi_{j,k-1} j a_2 + \varphi_{j,k+1}(k+1)d_2^x - \varphi_{j,k}(ia_1 + jd_1^x + ja_2 + kd_2^x). \tag{35}$$

We can define the average amount of each of the species x_1 and x_2 as

$$x_1 = \langle j \rangle = \sum_{j=0}^{\infty} \varphi_{jk} j, \quad x_2 = \langle k \rangle = \sum_{j=0}^{\infty} \varphi_{jk} k.$$

Equations for these quantities can be derived from Eq.35; they are

$$\dot{x}_1 = a_1 x_0 - d_1^x x_1,$$
$$\dot{x}_2 = a_2 x_1 - d_2^x x_2.$$

These are identical to Eqs. 5 and 6. Following Gillespie's argument (54), we can write down a continuous (diffusion) approximation of the master equation. Defining continuous variables X_1 and X_2 such that $\varphi_{jk}(t) = \varphi(X_1, X_2; t)$, and expanding the terms in the right-hand side of the master equation into a Taylor series up to the second order, we obtain the following Fokker-Planck equation:

$$\frac{\partial \varphi}{\partial t} = -\frac{\partial \varphi}{\partial X_1}[A_1 \varphi] - \frac{\partial \varphi}{\partial X_2}[A_2 \varphi] + \frac{1}{2}\frac{\partial^2 \varphi}{\partial X_1^2}[B_1 \varphi] + \frac{1}{2}\frac{\partial \varphi}{\partial X_2^2}[B_2 \varphi],$$
$$A_1 = X_0 a_1 - X_1 d_1^x, \quad A_2 = X_1 a_2 - X_2 d_2^x,$$
$$B_1 = X_0 a_1 + X_1 d_1^x, \quad B_2 = X_1 a_2 + X_2 d_2^x. \tag{36}$$

Here, A_1, A_2, B_1, and B_2 are the drift and diffusion coefficients, respectively. Equation 36, just like the master equation, describes the evolution of the probability distribution function, $\varphi(t)$. For individual stochastic trajectories, a different description has to be developed. Using the expressions for the drift and diffusion coefficients, we can derive the chemical Langevin equation for the stochastic variables:

$$dX_1 = A_1 dt + \sqrt{B_1} dW_1,$$
$$dX_2 = A_2 dt + \sqrt{B_2} dW_2, \qquad (37)$$

where W_1 and W_2 are statistically independent white noise sources. This equation can be derived without using the procedure of diffusion approximation, by simply expressing the first two moments of the change of variables X_1 and X_2 in terms of time dt. Note that these stochastic differential equations (SDEs) (Eq. 37), contain the deterministic (drift) part that is identical to that of the equations for the average values, x_1 and x_2. The diffusion part, which multiplies the white noise term accounts for the intrinsic noise in the system.

There are many ways to incorporate external noise in the system. However, to illustrate the effect of external noise, it suffices to use a simple description. Here we assume that the noise affects the variable x_0, the input signal, and that it propagates down the cascade by means of Eq. 37. The input signal can be thought of as the solution of the Ornstein-Uhlenbeck-type equation,

$$dX_0 = (\mu^x(t) - d_0^x X_0) dt + \sqrt{D} dW_0,$$

that is, in amount of species X_0 oscillates around a mean value, $\mu^x(t)$.

This derivation can be easily generalized to more complicated or even nonlinear networks. The nonlinearities in the activation-deactivation coefficients are simply carried over to the drift and diffusion terms. To give an example, we present the SDEs for two parallel cascades with a shared element and a nonlinearity:

$$dX_0 = (\mu^x(t) - d_0^x X_0) dt + \sqrt{D_x} dW_0^x,$$
$$dY_0 = (\mu^y(t) - d_0^y Y_0) dt + \sqrt{D_y} dW_0^y,$$
$$dX_1 = (a_1 X_0 + b_1 Y_0 - d_1^x X_1) dt + \sqrt{a_1 X_0 + b_1 Y_0 + d_1^x X_1} dW_1^x,$$
$$dX_2 = (a_2 R[X_0] X_1 - d_2^x X_2) dt + \sqrt{a_2 R[X_0] X_1 + d_2^x X_2} dW_2^x,$$
$$dY_2 = (b_2 X_1 - d_2^y y_2) dt + \sqrt{b_2 X_1 + d_2^y Y_2} dW_2^y.$$

Solutions of these equations have the form

$$X_1(t) = x_1(t) + \zeta_1^x, \quad X_2(t) = x_2(t) + \zeta_2^x,$$
$$Y_1(t) = y_1(t) + \zeta_1^y, \quad Y_2(t) = y_2(t) + \zeta_2^y,$$

where the lower case symbols denote the expected value of each variable and the terms $\xi_{1,2}^{x,y}$ are the stochastic parts, with some important properties that we will discuss.

Now we can introduce definitions of specificity and fidelity. Using a similar approach to that taken in the determin-

istic case, we define $\bar{X}_f|X = \int_0^\infty X_f(t)\, dt|_{x_0>0,y_0=0}$ as the total amount of final product X_f when the cell is exposed to signal x_0 but not to signal y_0. The specificity and fidelity of channel X are given by

$$S_X = \frac{\langle \bar{X}_f|X \rangle}{\langle \bar{Y}_f|X \rangle}, \quad F_X = \frac{\langle \bar{X}_f|X \rangle}{\langle \bar{X}_f|Y \rangle}, \qquad (38)$$

where the final output variables are in our case X_2 and Y_2. The triangular brackets denote the expected value of the corresponding quantity. The specificity and fidelity of other channel(s) are defined similarly.

To calculate the specificity of the X channel for the stochastic system, we first integrate the SDEs to obtain

$$X_2(\infty) - X_2(0) = a_2 \overline{X_1 R[X_0]} - d_2^x \bar{X}_2 + \int_0^\infty \sqrt{B_2^x} W_2 dt, \quad (39)$$

where the bar denotes the integration in time from zero to infinity, and the expression under the square root is the diffusion coefficient. Taking the average of Eq. 39, we can see that the left-hand side disappears, because on average the initial and the end concentration of the protein are assumed to be the same. Also, the term with the white noise disappears, because $\left\langle \int_0^\infty \sqrt{B_2^x} W_2 dt \right\rangle = 0$ by the Ito integration rule of nonanticipating functions (55). Thus, we have:

$$\langle \bar{X}_2 \rangle = \frac{a_2}{d_2^x} \langle \overline{X_1 R[X_0]} \rangle.$$

Similar expressions can be derived for the average amount of all species. In fact, these expressions are not different from the deterministic ones obtained previously. As a result the stochastic effects do not change the specificity and fidelity calculated using the deterministic approach. This is not surprising, because this study is concerned with global characteristics of the system, and the noise usually does not influence ensemble-averaged quantities.

Before we go on, we would like to comment on the averaging procedure used in the above definition. The specificity and fidelity are evaluated by first averaging both signals (under the condition that one input is on and the other is off), and then a ratio is formed. An alternative way would be to evaluate the following:

$$S_X = \left\langle \frac{X_f|X}{Y_f|X} \right\rangle, \quad F_X = \left\langle \frac{X_f|X}{X_f|Y} \right\rangle,$$

that is, the two procedures, evaluating the ratio and taking an average, are interchanged. The results are of course different for the two definitions; we would like to argue that the first definition makes more intuitive sense. Let us suppose that the output signal in the numerator is noisy and the one in the denominator is not. Then by above procedures we can see that the noise averages out and does not affect the overall result. Next, let us assume that the output signal in the denominator is noisy and the one in the numerator is not. Now,

the result of the averaging will be different. This means that in this definition, noise affects the numerator and the denominator differently. This is an undesired asymmetry. Intuitively speaking, the numerator and the denominator should be treated equally, because for all practical purposes an equally good measure of specificity can be defined with the numerator and the denominator reversed. Therefore, we conclude that our initial definition is a more suitable measure of specificity/fidelity in noisy systems.

Finally, a note on the limits of integration. Intuitively, the quantities that appear in the numerator and denominator of the definitions of specificity and fidelity, Eq. 38, are "total, ensemble averages" of the signals of interest. To calculate the total amount of the signal in each realization, we integrated the corresponding signal amplitude in time from zero to infinity. This is of course an idealization. In reality, an infinitely long time is a span of time that is longer than the characteristic time of the (deterministic) signal change. In some cases, this can be estimated as the inverse of the smallest eigenvalue of the (linearized) deterministic matrix governing the average behavior. In general, it is the time it takes for the system to settle near a steady state. This time, by definition, must be larger than any characteristic fluctuation time in the environment.

Inclusion of background signal levels and experimental measurements

In actual cell signaling networks, it is unusual for pathways to be completely "off", rather, there is some amount of basal signaling. That is, for most pathways, even if they are not receiving a signal, a small but significant fraction of the kinases are nevertheless active, and there is a low but significant level of expression of downstream target genes. This is certainly true of the pathways in the mating/invasive growth signaling network, for example. In this section we will first consider how basal signal levels can be taken into account in experimental measurements of specificity and fidelity, and then discuss how they can be handled within our formal mathematical framework.

For the output P_{out} of any pathway P, let us distinguish the basal or background signal level, P_b, and the signal-regulated part, P_s, so that $P_{out} = P_b + P_s$. P_b is essentially independent of the input signal and can be considered to be a constant, or to fluctuate around some constant average value. Of course, if P_b is small compared to P_s, then P_b can be ignored. If not, then, in experimental measurements, it will often be convenient to express output as a fold change with respect to the basal level, i.e.,

$$P_{out} = \frac{P_b + P_s}{P_b}.$$

Thus, with regard to an XY network of the type we have been considering in this article,

$$X_{out}|X_{in} = \frac{X_b + X_s|X_{in}}{X_b}, \quad X_{out}|Y_{in} = \frac{X_b + X_s|Y_{in}}{X_b}$$

$$Y_{out}|Y_{in} = \frac{Y_b + Y_s|Y_{in}}{Y_b}, \quad Y_{out}|X_{in} = \frac{Y_b + Y_s|X_{in}}{Y_b}. \quad (40)$$

Thus we have

$$F_X = \frac{X_{out}|X_{in}}{X_{out}|Y_{in}} = \frac{X_b + X_s|X_{in}}{X_b + X_s|Y_{in}};$$

$$S_X = \frac{X_{out}|X_{in}}{Y_{out}|X_{in}} = \frac{(X_b + X_s|X_{in})Y_b}{(Y_b + Y_s|X_{in})X_b}, \quad \text{etc.}$$

As can be seen, with these modified definitions of output, fidelity remains a number that is easily interpretable as the ratio of X output during authentic versus spurious signaling. Moreover, even if pathway X has complete fidelity ($X_s|Y_{in} = 0$), it will still have a finite fidelity (equal to its fold induction at a given level of authentic signal). Because fidelity by definition measures the response to two different signals, however, one problem that might arise experimentally concerns the appropriate levels of the different inputs.

Specificity avoids this problem, since there is only one input. However, since there are by definition two outputs, there is the problem of comparing apples to oranges. This is somewhat mitigated, however, by expressing output as a fold change with respect to the basal signal level (Eq. 40). With the modified output definitions, if S_X is >1 it means that X_{in} causes a greater fold change in X_{out} than in Y_{out}.

For a more formal treatment of basal signal levels, let us now consider the output $\mu(t)$ of pathway P at a given time during the time course of the signaling event. We have

$$\mu(t) = \mu_b + \mu_s(t),$$

where the first term on the right-hand side is some constant nonzero background level and the second term is the signal-regulated part, such that $\mu_s(0) = 0$ and $\lim_{t \to \infty} \mu_s(t) = 0$. As time tends to infinity, the steady-state quantities in our system do not tend to zero, and the operation of integration from zero to infinity in the definitions of specificity and fidelity becomes undefined. This technical problem with the definition can be solved in the simplest way by subtracting the background level of the output signal under the sign of the integral. That is we replace the expression $\int_0^\infty X_f(t)dt|_{x_0>0,y_0=0}$ with the expression $\int_0^\infty (X_f(t) - \lim_{t \to \infty} X_f(t))dt|_{x_0>0,y_0=0}$, and similarly for other integrals in the definition. Note that if the signal is much stronger than the background, then we have the following approximation:

$$\int_0^\infty (X_f(t) - \lim_{t \to \infty} X_f(t))dt|\{\mu(t) = \mu_b + \mu_s(t)\}$$

$$\approx \int_0^\infty X_f(t)dt|\{\mu(t) = \mu_s(t)\}.$$

In other words, instead of subtracting the background level of the output signal, we can simply consider a system where there is no background input signal. The smallness

of the background condition can be rigorously expressed as follows: there exists a time, T, such that $\int_0^T \mu_s(t)dt \gg \int_T^\infty \mu_s(t)dt$ and in the time interval $0<t<T$, we have $\mu_s(t) \gg \mu_b$. In other words, for most of the duration of the signal, it must be much larger than the background level.

In conclusion, the presence of a small (compared to the signal) constant background level does not change the properties of specificity and fidelity, where we assumed no such background level. A strong background level of signal requires a small modification on the original definition by subtracting the background level from the output signals to ensure the convergence of integrals.

DISCUSSION

A quantitative understanding of intracellular signal processing will substantially increase our comprehension of biological systems and may catalyze radical changes in how diseases are analyzed and treated (4,56). A major obstacle to this goal, however, is the challenge of obtaining a broad and integrated appreciation of the mechanisms that promote signaling specificity. The maintenance of specificity must have been a critical factor in the evolution of signaling networks, replayed each time a new pathway emerged by duplication and divergence of preexisting parts. Thus, the requirement for specificity has undoubtedly shaped the design logic of biochemical networks.

Here we calculated specificity and fidelity—two quantities that capture much of the intuitive biological concept of specificity—in simple stochastic signaling networks, and examined the performance of several specificity-promoting enhancements, or insulating mechanisms. This exercise supplied insight into the strengths and weaknesses of these insulating mechanisms, and exposed the constraints they may place on the properties and performance characteristics of the networks in which they are utilized. We also considered the effect of stochastic fluctuations (''noise'') in the levels of network proteins on specificity and fidelity, and showed that these metrics can be used even for noisy pathways.

Specificity and fidelity

Specificity and fidelity are simple ratios of pathway output that capture common sense notions that a pathway should stimulate its own output more than another pathway's output, and respond to its own input more than to another's. For small networks analyzed under simplifying assumptions, specificity and fidelity can be expressed as a function of key parameters of the network, as we have done here. For more complex, nonlinear models of signaling networks, specificity and fidelity can be readily calculated through numerical simulations (29). In fact, it should be possible to calculate pathway fidelities and network specificity from the results of experimental measurements made on cells or tissues.

The utility of the metrics specificity and fidelity is reinforced by the intuitively pleasing results obtained when they were calculated for simple networks. Here, a measurement that we termed network specificity, obtained by multiplying the pathway specificities or pathway fidelities together, proved to be informative. In the network consisting of two parallel pathways that did not share components, yet exhibited undesired cross talk due to imperfect kinase selectivity (Fig. 2 A), specificity and fidelity could be increased simply by increasing the selectivities of the transgressing kinases (Fig. 3). When a single kinase was shared between two pathways, however (Fig. 4 A), biasing the selectivity of that kinase for a substrate in one pathway increased that pathway's specificity, but correspondingly decreased the specificity of the other pathway. The specificity of this network (which we dubbed the basic architecture) turned out to be constant, and equal to unity. Thus, network specificity could not be achieved by altering kinase selectivities (nor by changing other parameters such as signal strength or deactivation rates). Achieving specificity in this case required embellishing the basic network architecture by the addition of an insulating mechanism.

Analysis of common insulating mechanisms

We next analyzed the properties of insulating mechanisms frequently found in biological signaling networks, asking to what extent they could enhance the specificity and fidelity of the basic architecture. Combinatorial signaling (Fig. 4, B and C) relies upon the ability of a target of one of the pathways to act as a coincidence detector or ''AND gate'' that integrates the signal from the shared kinase with a second input that is not shared. If pathway X received the combinatorial input, then the fidelity of pathway X, the specificity of pathway Y, and network specificity could all be increased by a factor equal to the reciprocal of the basal strength of the second input. Cross-pathway inhibition (Figs. 4 D and 6), modeled after recent findings in yeast (57), occurs when a downstream component of one pathway inhibits the target of the other pathway. Cross-pathway inhibition of X by Y was effective in increasing the specificity and fidelity of pathway X, but created problems with the fidelity of pathway Y.

In compartmentalization (Fig. 4 E), the two pathways are localized to different parts of the cell, and leaking only occurs to the extent that the shared kinase moves between the two compartments. This mechanism could increase the specificity and fidelity of both pathways to the extent that deactivation of the shared kinase was faster than its diffusion (28).

The final insulating mechanism we examined was scaffolding (Figs. 4 F and 8). By binding to multiple consecutively acting components of a pathway, a scaffold protein can sequester one pathway from another in a manner similar to compartmentalization, but at a smaller spatial scale. Scaffolding could significantly increase the specificity and

fidelity of both pathways if the shared kinase was deactivated faster than it moved on/off the scaffold (28). Adding selective activation of the scaffold (where the scaffold is only capable of productively binding the shared kinase during authentic signaling) could further increase the specificity of the unscaffolded pathway and hence the network by a mechanism resembling combinatorial signaling.

The yeast mating/invasive growth network contains several of these insulating mechanisms. Some predictions emerging from our analysis of simple mathematical models appear to provide useful insights into how specificity is obtained in this biological system, as will be highlighted in the next two sections.

Constraining effects of insulating mechanisms

The specificity mechanisms we examined were unable to provide mutual specificity and fidelity to both pathways in a network without constraining the network by placing additional requirements on parameters values. For example, compartmentalization only worked well if deactivation of the shared kinase was faster than the movement of the kinase between compartments; thus compartmentalization constrains deactivation rates not to be too slow (58). It has been argued that kinase dephosphorylation rates are indeed fast relative to diffusion (59,60). Likewise, for scaffolds to effectively promote specificity by sequestration, deactivation rates must be fast. Furthermore, scaffold binding/dissociation must be slow, which is likely to constrain signal speed and amplification.

If pathway X received a combinatorial input, it exhibited increased fidelity, and pathway Y benefited from increased specificity. Mutual fidelity, however, required a greater amplitude and/or duration of the Y signal relative to the X signal. Moreover, mutual specificity in such a network could only be obtained if the X-pathway target was more sensitive than the Y-pathway target to the shared kinase (Fig. 5).

Cross-pathway inhibition (X inhibits Y) was the most constraining insulating mechanism of all, and overall, the least effective. Mutual specificity could only be achieved if the Y-pathway target was more sensitive than the X-pathway target to the shared kinase. Moreover, mutual specificity and fidelity could only be obtained if the Y signal was weaker, but substantially longer, than the X signal (Fig. 7). Interestingly, this does seem to be true of the yeast mating/invasive growth network, where cross-pathway inhibition from mating (X) to invasive growth (Y) is employed (Fig. 6). Invasive growth-promoting conditions provide a weak signal presumed to last for days, whereas physiological levels of mating pheromone supply a much stronger signal that lasts only a few hours (18).

It is likely that in real biological networks, some of these constraints are mitigated by adding a second insulating mechanism, for example, by providing a combinatorial input to both pathways. The yeast mating/invasive growth network features two complementary insulating mechanisms. The first mechanism is, as mentioned above, is cross-pathway inhibition: Fus3$^{\mathrm{MAPK}}$ (mating pathway) inhibits the Tec1 transcription factor (invasive growth), which serves primarily to decrease leaking from the mating pathway into the invasive growth pathway. The second mechanism is scaffolding: Ste5 is a selectively activated scaffold for the mating pathway that functions primarily to decrease leaking from the invasive growth pathway into the mating pathway (23). These two insulating mechanisms appear to complement each other reasonably well: cross-pathway inhibition from mating (X) to invasive growth (Y) is predicted to increase S_X but reduces S_Y and F_Y, whereas a selectively activated scaffold for the mating pathway (X) would increase S_Y (and slightly increase F_Y, given that the exchange rates D_{out} and D_{in} appear to be rather high, as discussed below).

What about desirable cross talk?

Although we have emphasized the minimization of undesirable signal crossover, there are many instances where cross talk between pathways is a useful and important means to integrate multiple signals (2). Our definitions provide a measure of cross talk, whether that cross talk is considered advantageous or disadvantageous (for other recent approaches to quantitatively evaluate cross talk see (29,61–63)). Thus, for example, our framework is also useful for the analysis of networks where cross-talk in one direction is desirable but leaking in the reciprocal direction is not. Such situations may be fairly common; if so, this may explain why pathways that share components are so widespread. In such cases network specificity is not a particularly useful concept, but pathway specificity and fidelity are. For example, if cross talk from pathway X to pathway Y is desirable, but leaking from Y to X is not, then evolution may have favored the selection of rate constants and insulating mechanisms that maximize S_Y and F_X while keeping S_X and F_Y near 1. One way this could be accomplished is by combinatorial signaling for X combined with near symmetric network parameters.

A concrete example of this phenomenon is found in yeast, where activation of the mating pathway leads to the phosphorylation and activation of both the Fus3 and Kss1 MAP kinases (18), whereas only Kss1 is activated during invasive growth (40). The phosphorylation of both MAP kinases contributes to the mating endpoint, but phosphorylation of Fus3 during invasive growth is undesirable, because Fus3 inhibits this process. Hence, if the mating pathway is X and the invasive growth pathway is Y, and if Fus3 is x_2 and Kss1 is y_2, then the network is structured such that S_X is close to 1; that is, Fus3 and Kss1 are activated to roughly equal extents during mating. However, S_Y appears to be much greater than 1; that is, Kss1, and not Fus3, is activated during invasive growth. As can be seen from Eqs. 28 and 33, this outcome could be accomplished by a selectively activated scaffold that had relatively high

values of D_{out} and D_{in} and a reasonably small value of k_{leak}. In fact, this situation is quite close to that suggested by our recent experiments (23).

Future directions

Here we concentrated on weakly activated pathways that can be modeled as linear systems because of their analytical tractability. In the Supplementary Material we demonstrate that, for strongly activated pathways, the basic architecture cannot exhibit mutual fidelity, and can exhibit mutual specificity only under certain restrictive conditions. However, other relationships between input and output should also be explored, such as ultrasensitivity and bistability (64–66). It seems possible that some of the insulating mechanisms will show improved performance under such conditions. Indeed, a fully nonlinear model of cross-pathway inhibition, explored computationally by Somsen et al., exhibits both mutual specificity and mutual fidelity in the steady state (67). Hence, a challenge for the future is to understand how feedback and ultrasensitivity influence specificity in interconnected biochemical networks.

Conclusions—design principles in pursuit of specificity

Are there common features or emergent properties of signaling networks that exhibit a reasonable degree of specificity despite undesired signal crossover? Although much more work will be required before this question can be completely answered, some hints have emerged from our analysis. First, networks in which different pathways share components will likely contain one or more insulating mechanisms that enhance specificity and fidelity. Second, more than one insulating mechanism will probably be required to provide a reasonable degree of mutual specificity and fidelity without placing significant constraints on the network. Third, constraints to be expected are differential target sensitivity, a requirement for fast deactivation, and a greater amplitude or/and duration of one signal relative to the other.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

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