Signal Processing in the TGF-β Superfamily Ligand-Receptor Network

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The TGF-β pathway plays a central role in tissue homeostasis and morphogenesis. It transduces a variety of extracellular signals into intracellular transcriptional responses that control a plethora of cellular processes, including cell growth, apoptosis, and differentiation. We use computational modeling to show that coupling of signaling with receptor trafficking results in a highly versatile signal-processing unit, able to sense by itself absolute levels of ligand, temporal changes in ligand concentration, and ratios of multiple ligands. This coupling controls whether the response of the receptor module is transient or permanent and whether or not different signaling channels behave independently of each other. Our computational approach unifies seemingly disparate experimental observations and suggests specific changes in receptor trafficking patterns that can lead to phenotypes that favor tumor progression.

Introduction

The TGF-β signal transduction pathway follows an apparently straightforward downstream cascade, progressing sequentially from the interaction of ligands with transmembrane receptors, through phosphorylation of mediator Smad proteins, to transcriptional responses (Figure 1). The simple logic of this signal transduction cascade strongly contrasts with the molecular complexity of the cellular processes involved and the wide diversity of responses triggered.

At the molecular level, there is an intricate signal transduction machinery that integrates signals from the 42 known ligands of the TGF-β superfamily, funnels them through the two principal regulatory Smad (R-Smad) channels (Smad1/5/8 or Smad2/3), and subsequently leads to the widespread transcriptional control of more than 300 target genes in a cell-context dependent manner [1] (see Figure 2). The components of this machinery include the members of the two main receptor families (type I and type II receptors), a myriad of adaptor proteins, and the trafficking apparatus of the cell, which shuttles proteins between different subcellular compartments. Each ligand induces the formation of a receptor complex with type I and type II receptors, which then signal through one of the two Smad channels [2,3]. The ability of most ligands to bind several type I and type II receptors results in a complex ligand-receptor interaction network (Figure 2).

At the phenotypic level, the responses are extremely diverse. The members of the TGF-β superfamily act prototypically as potent negative growth regulators, but, depending on the cell type and context, they can also induce differentiation, apoptosis, cell migration, adhesion, and extracellular matrix deposition. TGF-β itself is of particular interest in cancer research. In epithelial cells, it suppresses cellular growth by inducing G1 arrest (mediated by transcriptional activation of p15 and p21) [4], and its inactivation contributes to tumorigenesis. The versatility of the pathway in eliciting different types of behavior is perhaps best epitomized by the pervasive, rather paradoxical ability of TGF-β to change its function from suppressor to promoter of growth in epithelial cells during tumor progression [4,5].

Current theories for explaining the variety of responses to members of the TGF-β superfamily of ligands focus mainly on the downstream transcriptional regulatory networks they activate: transcriptional cofactors of the R-Smads are expressed at different levels in a cell-specific manner, thereby modifying downstream responses. In fact, the role reversal of TGF-β from negative to positive growth regulator has been found to be associated with a phenotypic change known as epithelial-to-mesenchymal transition, in which cells change the cofactors recruited by the R-Smads and acquire motile phenotypes [5–7].

It is striking, however, that such a variety of complex responses and intricate molecular components are connected through just two Smad channels by such a simple downstream signal transduction cascade. There is a richness of experimental observations that are difficult to reconcile with this observation. In particular, whether TGF-β acts as a growth suppressor or promoter can depend on whether the tumor cells were grown in vitro or in vivo [8]. In these two different situations, the extracellular context determines the way in which cells respond to TGF-β. It has been suggested that TGF-β can suppress the growth of cells around the tumor, that it can shut down locally the immune system, and that it can...
Promote angiogenesis. All these paracrine effects would help
the growth of the tumor in vivo, where it has to compete with
neighboring cells. So far, although appealing, none of these
mechanisms has been identified as an alternative cause of the
TGF-β role reversal.

The most direct way in which the extracellular context can
affect the functioning of the TGF-β pathway is through
signaling of other ligands of the TGF-β superfamily. As we
have mentioned, ligands and receptors form a complex
interaction network, where multiple ligands share receptors,
potentially coupling their signaling. All these interactions are
in turn coupled to receptor trafficking, which is known to be a
mechanism that regulates signal transduction [9,10]. Traffick-
ing has been investigated in detail in many signal trans-
duction pathways, such as the epidermal growth factor
receptor (EGFR) and G protein-coupled receptor (GPCR)
pathways [11–13]. The typical way in which trafficking and
signaling are coupled is by the induction of receptor
internalization upon ligand binding and receptor activation,
as for instance in the EGFR and GPCR pathways. After
internalization, receptors can activate other signaling path-
ways, be modified in specific ways, and be targeted for
degradation or recycling back to the plasma membrane.

A peculiarity of the TGF-β pathway is that receptors are
constitutively internalized, even in the absence of ligand
[14,15]. The trafficking route that the receptors follow,
Signal Processing in the TGF-β Pathway

Receptors in the plasma membrane interact with the signaling peptides of the TGF-β superfamily to form active complexes. Receptors and activated ligand-receptor complexes can internalize via clathrin-coated pits into endosomes, from where the active ligand-receptor complexes phosphorylate the cytoplasmic R-Smads (“receptor Smads,” either the Smad1/5/8 or the Smad2/3 group) [33]. The phosphorylated R-Smads form complexes with the Co-Smad (Smad4) and then translocate into the nucleus where they act as transcriptional regulators of about 300 target genes.

The internalized receptors recycle back to the plasma membrane (with a characteristic time of ~30 min) via a rab11-dependent, rab4-independent pathway [14]. After returning to the plasma membrane, the receptors that were actively signaling can be targeted for degradation or be used for further ligand-binding or internalization [14]. Receptors that did not bind ligands are simply returned to the plasma membrane. As a consequence of the trafficking processes, only about 5%-10% of receptors are present in the plasma membrane [15]. In addition to the traditional clathrin pathway, active ligand-receptor complexes can recruit Smad7-Smurf2 [28], which then targets them to lipid raft–caveolar compartments (right) for degradation [15]. The ligands do not return back to the plasma membrane, but disassociate from the receptors before recycling and undergo direct degradation via the lysosomes [14].

Note that, in addition to the ligand-induced receptor degradation, we also consider a receptor degradation pathway that functions independently of ligand-binding; this represents a “constitutive” or ligand-independent degradation pathway (left).

DOI: 10.1371/journal.pcbi.0020003.g003

Results

A Concise Computational Model

In order to study the signal processing potential of the ligand-receptor network coupled with receptor trafficking, we assemble all the essential elements into a concise mathematical model that captures the logic of the underlying processes. The main goal is to represent as much complexity as possible through a small number of quantities that have direct experimental interpretation.

The essential elements we consider are (Figure 4): (i) Ligands induce the formation of receptor complexes with type II and type I receptors. (ii) Receptors and ligand-receptor complexes can be present in two spatially distinct compartments: plasma membrane and internalized endosomes. (iii) The signaling activity is proportional to the number of ligand-receptor complexes that are present in the internalized endosomes [16]. (iv) Receptors and ligand-receptor complexes are continuously internalized into endosomes and recycled back to the plasma membrane [14, 15]. (v) Receptor degradation has a constitutive contribution, which is the same for free receptors and ligand-receptor complexes [14,15]. (vi) Receptor degradation has a ligand-induced contribution, which affects only receptors that have been complexed with ligands [14,15].

We use these elements to develop a mathematical model based on rate equations that describe the dynamics of both how different molecular species transform into each other and how they traffic between the different cellular compartments. We assume that internalization, recycling, and degradation rates are proportional to the number of receptors or ligand-receptor complexes; and that the formation of ligand-receptor complexes is proportional to the ligand concentration and to the type I and type II receptor concentrations.

In a first step toward characterizing the effects of the coupling of signaling with receptor trafficking, we consider that only a single type of ligand is present. Explicitly, we study how the components of the canonical TGF-β pathway—one ple ligands that can be passed downstream independently of or dependently on each other.

A sustained response implies that the steady-state signaling activity is a function of the ligand concentration. In this case, the higher the ligand concentration, the higher the activity of the pathway. For a transient response that precisely returns to the prestimulus level, in contrast, the steady-state activity is always the same and the pathway can only detect changes in ligand concentration. When multiple ligands signal in a dependent fashion, the extent of the coupling can be such that one ligand can suppress the effects of another one. In this regime, the pathway does not detect ligand concentrations but ratios of concentrations.

As we show here, all these types of behavior can be present in the TGF-β pathway. Which specific one is selected is determined by the interplay between trafficking and signaling. Thus the pathway can be set to detect, at the receptor level, absolute levels of ligand, temporal changes in ligand concentration, and ratios of multiple ligands. Such flexibility in the pathway behavior can lead to diverse physiological outcomes that have been associated with facilitated tumor progression.

Figure 3. Signaling and Trafficking in the TGF-β Pathway

Receptors in the plasma membrane interact with the signaling peptides of the TGF-β superfamily to form active complexes. Receptors and activated ligand-receptor complexes can internalize via clathrin-coated pits into endosomes, from where the active ligand-receptor complexes phosphorylate the cytoplasmic R-Smads (“receptor Smads,” either the Smad1/5/8 or the Smad2/3 group) [33]. The phosphorylated R-Smads form complexes with the Co-Smad (Smad4) and then translocate into the nucleus where they act as transcriptional regulators of about 300 target genes.

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DOI: 10.1371/journal.pcbi.0020003.g003

Here we characterize computationally the diverse potential types of behavior that the pathway structure itself can confer on the system. The types of behavior include responses to persistent changes in ligand concentration that can be transient or sustained and simultaneous responses to multiple ligands that can be passed downstream independently of or dependently on each other.

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In a first step toward characterizing the effects of the coupling of signaling with receptor trafficking, we consider that only a single type of ligand is present. Explicitly, we study how the components of the canonical TGF-β pathway—one
ligand (TGF-β) and two receptors (Alk5 and TGFβRII), as emphasized in Figure 2—respond to changes in ligand concentration. The mathematical equations are shown in Figure 4. For typical trafficking rates (see Materials and Methods), this model closely reproduces the typical time courses of Smad phosphorylation upon addition of ligand (Figure 5A and 5B).

The computational model can be used also to analyze how different parameters affect the behavior of the system. For instance, the time during which the signaling activity rises is related to the time required for internalization and recycling of the receptors. Thus, the signal will peak, or stop rising, at about 30–60 min after ligand addition. If the internalization and recycling rates are changed, the position of the peak changes accordingly (Figure 5C and 5D).

Other transmembrane receptor pathways, such as the EGFR pathway, have much faster kinetics; the EGFR pathway reaches peak activity as fast as 5 min after stimulation [17]. The main reason for these differences is that most of the EGF receptors are present in the plasma membrane and they are ready to signal upon the addition of ligand. A similar kinetics is also observed for many GPCRs. In the TGF-β pathway,
The typical response to sustained changes in TGF-β concentration shows partial adaptation after reaching a maximum of activity. Different values of the parameters of the model lead to this characteristic behavior. In all panels, the TGF-β concentration is increased at time 0 to saturating values and kept constant afterward, as in inset (A).

(A) Behavior of the model for typical trafficking rates: internalization, $k_i = (3 \text{ min})^{-1}$; recycling, $k_r = (30 \text{ min})^{-1}$; constitutive degradation, $k_{cd} = (36 \text{ min})^{-1}$; ligand-induced degradation $k_{ld} = (4 \text{ min})^{-1}$; efficiency of recycling of active receptors, $\alpha = 1$. Note that the trafficking rate constants are given as the inverse of the corresponding characteristic times. The production of receptors is chosen to be $p_R = 8 \text{ min}^{-1}$ and $p_W = 4 \text{ min}^{-1}$, which leads to $10^3$ receptors per cell under stationary conditions in the absence of ligand. The units of ligand concentration are chosen so that the association rate constant is the unit, $b = 1$.

(B) Comparison of the model time course (upper lane) with an experimental time course of nuclear phosphorylated Smad2 (P-Smad) as reported by Inman et al. (bottom lane) [16]. The model results from (A) are shown at the time course of nuclear phosphorylated Smad2 (P-Smad) as reported by Inman et al. (bottom lane) [16]. The signal peaks at approximately 60 min.

(C) Behavior of the model with the same parameter values as in (A), with the exception of the rate constants for internalization and recycling that have been decreased to $k_i = (10 \text{ min})^{-1}$ and $k_r = (100 \text{ min})^{-1}$. The signal peaks at around 180 min.

(D) Behavior of the model with the same parameter values as in (A), with the exception of the rate constant for ligand-induced degradation that has been increased to $k_{ld} = 0$ and the efficiency of recycling of active receptors that has been decreased to $\alpha = 0.5$. This implies that ligand-receptor complexes are not degraded via the caveolae pathway. In contrast, 50% of the active ligand-receptors that come back to the plasma membrane after they have signaled are degraded.

(E) Behavior of the model with the same parameter values as in (A), with the exception of the rate constant for ligand-induced degradation that has been decreased to $k_{ld} = 0$ and the efficiency of recycling of active receptors that has been decreased to $\alpha = 0.5$. This implies that ligand-receptor complexes are not degraded via the caveolae pathway. In contrast, 50% of the active ligand-receptors that come back to the plasma membrane after they have signaled are degraded.

(F) Behavior of the model with the same parameter values as in (A), with the exception of the efficiency of recycling of active receptors that has been decreased to $\alpha = 0.5$. These parameters account for both types (caveola and recycling-dependent) of ligand-induced degradation.

DOI: 10.1371/journal.pcbi.0020003.g005

Control of the Signal: Transient versus Permanent Responses

How is it possible to modify the form in which the system responds to changes in TGF-β concentration? A mathematical analysis of the model (see below and Materials and Methods for details) indicates that the key quantity that determines the qualitative behavior of the pathway is the ratio of the constitutive to the ligand-induced rate of degradation, referred to, in short, as the constitutive-to-induced degradation ratio (CIR). This quantity compares the rates of two degradation processes and, in general, does not have a simple expression in terms of rate constants.

Depending on the CIR, a permanent change in ligand concentration can elicit responses between two extremes (Figure 6). For low CIR, the ligand-induced degradation process dominates and there is a transient increase in signaling activity that returns to pre-stimulus levels (Figure 6A and 6B). For high CIR, the constitutive degradation process dominates and there is a permanently elevated level of signaling activity (Figure 6E and 6F).

For intermediate CIR, the behavior of the system is a mixture of both limiting types of behavior, with transient and permanent components (Figure 6C and 6D). The precise parameter values influence the amplitude and characteristic time of the response (see for instance Figure 5C and 5D), but its qualitative shape, that is, whether the response is transient (Figure 6A and 6B) or permanent (Figure 6E and 6F), depends only on the CIR.

The intuitive explanation of such types of behavior is as follows (for a detailed mathematical analysis, see Materials and Methods). The probability for a receptor to bind the ligand, and therefore to become active, increases with the ligand concentration. If the ligand does not induce the degradation of receptors, the number of receptors remains constant.
constant and the total activity increases when the ligand concentration increases. If the ligand induces the degradation of the receptors, the number of receptors starts to decrease after ligand addition, which will eventually attenuate the signal. At steady state, the production and degradation of receptors equal each other. In the limit of the CIR going to 0, the signal adapts completely because degradation is proportional to the activation of receptors, and therefore activation is also proportional to the production of receptors. Thus, it is the receptor production rate, not the ligand concentration, that determines the steady-state signaling activity.

There are clear examples in other signal transduction pathways that show that these two limiting types of behavior can potentially lead to different physiological outcomes. For instance, transient activation of the MAPK cascade by EGF leads to cell proliferation. In contrast, permanent activation of the MAPK cascade by NGF leads to cell differentiation. In both cases, activation of the MAPK cascade induces the expression of a negative regulator that shuts down the activity of this cascade. The differences between EGF and NGF have been attributed to additional pathways activated by NGF that can prevent the inactivation of the MAPK cascade [18]. Our model shows that such transient and permanent types of behavior can also be achieved by just changing the trafficking patterns, in particular by adjusting the CIR, without the need for explicitly expressing a negative regulator to shut down the cascade after signaling.

Remarkably, the duration of the signaling activity also seems to affect the physiological outcomes triggered by TGF-β [19]. Epithelial cells that are sensitive to the antiproliferative effects of TGF-β (HaCaT and Colo-357) have sustained activity of more than 6 h. In contrast, pancreatic tumor cell lines (PT45 and Panc-1) show short transient activity of about 1–2 h. Such a short transient confers resistance to the antiproliferative effects of TGF-β but maintains other responses to TGF-β that can lead to increased malignancy and invasiveness [19]. In our model, those differentiated types of behavior arise naturally for different trafficking patterns. In particular, short transients and sustained responses imply a low and a high CIR, respectively.

Control of the Signals: Coupled versus Uncoupled Channels

In vivo conditions, in contrast to those typical of in vitro experiments, expose cells to complex environments with many different growth factors. When multiple ligands of the TGF-β superfamily are present at the same time, they are likely to affect each other’s signaling (Figure 2). To study how multiple simultaneous input signals are integrated into coordinated transcriptional responses, we extend our computational model to consider two ligands that signal through two different type II receptors and a shared common type I receptor (see Materials and Methods for the mathematical equations). This is the simplest case of signal integration.

Intuitively, one should expect signals to be coupled when the shared receptor is saturated with ligands and uncoupled when ligand concentrations are low. At saturation, increasing the concentration of one ligand, and thus the concentration of the corresponding ligand-receptor complex, will take the shared receptor away from the complex formed by the other ligand, thus decreasing its signaling.

A mathematical analysis of the model (see Materials and Methods) indicates that even when the receptors are far from ligand-saturating conditions, it is possible for signals to affect each other. The key element is again receptor trafficking. In essence, the coupling arises because the induction of degradation of the common receptor by one ligand attenuates the effects of the other ligand, which also requires the common receptor to transduce the signal.

For pathways working away from receptor saturation, the interplay between trafficking and signaling determines how multiple simultaneous signals are passed downstream. As in the single ligand case, there are two extreme types of behavior (Figure 7): For low CIR, the ligand-induced degradation process dominates and signals are completely coupled (Figure 7A and 7B). For high CIR, the constitutive degradation process dominates and signaling is uncoupled (Figure 7E and 7F).
Figure 7. Control of Signal Integration

Time courses of the numbers of active receptor complexes when TGF-β concentration (in red) is increased, repeatedly in steps or continuously. Left panels (A, C, and E) show the responses to step increases as shown in inset in (A). Right panels (B, D, and F) show the response to a continuous increase as shown in inset in (B). There is also a second ligand present (here BMP7, in blue) whose concentration is kept constant. The two ligands induce the formation of two ligand-receptor complexes, CBMP7 (blue) and CTGF (red), that share the type I receptor Alk2. The green line on the left panels shows the total number of active receptor complexes (CBMP7 + CTGF). As in Figure 6, a key control quantity of the qualitative behavior of the system is the CIR. The parameter values for (A–F) are the same as in Figure 6A–6F, respectively.

When the signals are completely coupled, the steady-state number of all ligand-receptor complexes remains constant and is independent of the ligand concentration. In this case, increasing one signal will decrease the other one by the same amount. When signals are uncoupled, the numbers of each species of ligand-receptor complexes change independently of each other. In general, for intermediate CIR, signals will affect how TGF-β is ultimately coupled to the cell cycle. Our model explicitly shows that the role reversal is a potentially intrinsic consequence of the design of the ligand-receptor interaction network and trafficking machineries and that it could be the result of TGF-β attenuating the effects of growth-suppressing signals of other members of the TGF-β superfamily that might be present in the in vivo cell environment.

Simultaneous Perfect Adaptation and Coupled Signaling

Our model also indicates that the conditions that give rise to completely coupled integration of multiple signals are the same that, in a single-ligand system, cause the signaling activity to completely adapt to its prestimulus level. Remarkably, this concurs with observations in prostate cancer cell lines, which show that the in vivo context can not only make TGF-β a growth promoter but also that the in vitro response to TGF-β is transient [8]. One should expect the extracellular environment of growth factors to be more complex in vivo than in vitro. This relationship between in vivo and in vitro behavior and its connection to the coupling between receptor trafficking and signaling underscores the importance of understanding how signal transduction pathways are embedded within the cellular microenvironment under physiologically relevant conditions. Not only mutations in the canonical pathway but also changes in trafficking patterns can move the pathway to a different functioning point.

The qualitative results of our model, such as the regimes leading to transient and permanent responses as well as to completely coupled and uncoupled modes of signal integration, do not depend on the details of the model but on general properties (see Materials and Methods). Thus the
main ideas are also relevant to other signal transduction pathways that are coupled to receptor trafficking. In particular, revisiting the experimental data, one can see that the interplay between adaptation and signal integration (Materials and Methods) is also present in the EGFR pathway (see Figures 4 and 5 of reference [23]), in which down-regulation of erbB-2 by EGF concurs with adaptation of the signal transmitted by EGFR.

**Discussion**

Cellular functions are controlled by networks of interacting molecules that operate at different levels of organization [24-26]. Here, we have developed a concise computational model of the TGF-β pathway that shows that the receptors for the TGF-β superfamily of ligands are not just passive signal transducers. They are organized in a network that is able to process the signals before passing them downstream. Changes in receptor trafficking patterns can modify the type of behavior of the pathway in response to single and multiple ligand inputs. Already at the receptor level, the pathway can detect absolute levels of ligands, temporal changes in ligand concentration, and ratios of multiple ligands. This extra level of regulation can explain a wide variety of phenomena, such as the counterintuitive role reversal of TGF-β from suppressor to promoter of growth, and leads to a unified interpretation of seemingly disparate experimental observations. A key quantity that determines the qualitative behavior of the pathway is the CIR of the receptors. For low CIR, the pathway responds transiently to sustained changes in ligand concentration, and the signaling activities of multiple simultaneous ligands become dependent on each other. Ligand-induced degradation is thus not only a mechanism for achieving transient responses and perfect adaptation, but also for coupling multiple signals.

Various experiments can be designed to test the predicted types of behavior. The most direct evidence would come from biochemical measurements of ligand-induced and constitutive degradation rates [14,15]. The observed degradation rates can then be related to measurements of the levels of signaling activity. For instance, decreasing ligand-induced degradation by blocking the lipid raft–caveolar pathway with nystatin, as in reference [15], should bias the system behavior toward persistent responses to step changes in ligand concentration. Molecular interventions, such as RNAi against the mRNA of proteins involved in the trafficking processes, and their effects on signaling activity would provide indirect evidence that can be related to the details of the model. Because of their well-established role in intracellular trafficking, small GTPase rab proteins [27] are prime candidates for this type of approach. Certain experimental observations [8,19] in conjunction with our model suggest that cells with phenotypes that favor tumor progression have low CIR. Further experiments could test the extent of this correlation by comparing trafficking rates and signaling activity between different cancerous and non-cancerous cell lines.

**Materials and Methods**

**Internalization rate.** It has been reported in Table 1 of reference [15] that after 15 min of labeling the receptors at the plasma membrane, only 8%, 6%, 4%, and 2% of the labeled receptors remain at the plasma membrane (the different percentage values correspond to different experimental conditions). The remaining labeled receptors have been internalized in either caveolin-1 positive or caveolin-1 negative vesicles. By using the formula \( k_i = -\frac{1}{\ln(2)} f_i \) where \( k_i \) is the internalization rate and \( f_i \) the fraction of labeled receptors that remain at the plasma membrane after a time \( t \), we obtain internalization rates of 1/5.9, 1/5.3, 1/4.7, and 1/3.8 min\(^{-1} \) respectively. We have chosen \( k_i = 1/(3 \text{ min}) \) for comparison with experimental data in Figure 5B.

Figure 4 of reference [14] shows that 1.7% of the total number of receptors is internalized per minute. When this value is rescaled by the fraction of receptors in the plasma membrane, it translates into 18% of surface receptors internalized per minute. This implies that this internalization rate is \( k_i = 1/(5.3 \text{ min}) \), which is similar to the results obtained from reference [15]. The details of this rescaling are as follows. Mathematically, the internalization rate constant is defined as \( k_i = \frac{N_{\text{sur}}}{N_{\text{tot}}} c \) where \( N_{\text{sur}} \) is the number of receptors at the plasma membrane, \( k_i \) the internalization rate. The fact that most of the receptors are internalized, so that \( N_{\text{sur}} \approx 0.1 N_{\text{tot}} \), leads to \( k_i = 10^{-3} k_{\text{Mitchell}} \). The factor 0.1 results from the fact that the internalization rate is about 10X higher than the recycling rate. Therefore, under stationary conditions, the number of internalized receptors is 10X higher than the number of receptors at the plasma membrane.

**Ligand-induced degradation rate.** Active ligand-receptor complexes in lipid raft–caveolar compartments can recruit Smad7–Smurf2 [28], which then targets them for degradation [15]. Reference [28] measured the constitutive degradation rate of Smad2 [28], which then targets them for degradation [15]. Reference [14] measured the constitutive degradation rate of Smad2 [28], which then targets them for degradation [15]. Reference [14] measured the internalization rates of light and heavy ERBB2 receptors in lipid raft–caveolar compartments with similar rates. We have chosen \( k_{\text{tid}} = 1/(4 \text{ min}) \) for comparison with experimental data in Figure 5B.

**Constitutive degradation rate.** When the lipid raft–caveolar pathway is blocked with nystatin, only \( 30\% \) of the initially labeled receptors remain in the cell after 8 h. This gives a characteristic degradation time of \( 400 \text{ min} \) with respect to the total number of receptors. Rescaling this number to the plasma membrane receptors, we obtain \( k_{\text{cd}} = -\frac{1}{\ln(0.3)} k_{\text{Mitchell}} \approx 1/(36 \text{ min}) \) for comparison with experimental data in Figure 5B.

**Recycling rate.** Reference [14] shows that after about 30 min cells stop secreting internally labeled TGF-β receptors. This recycling rate is similar to that for the EGF receptor. We have chosen \( k_r = 30 \text{ min} \) for comparison with experimental data in Figure 5B.

**Steady and quasi-steady state analysis.** Here we study mathematically the properties of the steady state of the system with a single ligand. By equating to 0 the derivatives in the model equations of Figure 4, we obtain that the steady-state number of internalized ligand-receptor complexes is

\[
[\text{IR}]_t[\text{IR}]_t = k_i \frac{k_{\text{tid}}[\text{IR}]_t[\text{IR}]_t}{k_i + k_{\text{tid}} + k_T},
\]

where the steady-state number of type I and type II receptors at the plasma membrane are obtained by solving the equations

\[
0 = a_I - c[\text{IR}][\text{IR}][\text{IR}]_t - [\text{IR}],
\]

\[
0 = a_{II} - c[\text{IR}][\text{IR}][\text{IR}]_t - [\text{IR}],
\]

with

\[
a_I = \frac{\alpha}{k_{\text{tid}}, \ a_{II} = \frac{\alpha}{k_{\text{tid}}} \alpha, \text{ and } c = \frac{k_{\text{tid}} + k_{\text{tid}} + (1 - \alpha) k_{\text{tid}}}{k_{\text{tid}} + k_{\text{tid}} + k_{\text{tid}}}/k_{\text{tid}}.}
\]

The solution of these equations is

\[
[\text{IR}]_t = \frac{a_I - a_{II}}{2} + \frac{\sqrt{1 + 2(a_I - a_{II})c + (a_I - a_{II})^2c^2 - 1}}{2c}
\]

\[
[\text{IR}]_t = \frac{a_{II} - a_I}{2} + \frac{\sqrt{1 + 2(a_{II} - a_I)c + (a_{II} - a_I)^2c^2 - 1}}{2c}
\]

which leads to

\[
[\text{IR}]_t = \frac{k_{\text{kd}}(1 + a_I + a_{II}c - \sqrt{1 + 2(a_{II} + a_I)c + (a_{II} + a_I)^2c^2})}{k_i(k_{\text{tid}} + k_{\text{tid}} + (1 - \alpha) k_{\text{tid}})}
\]

For low values of \( c \), Equation 5 reduces to

\[
[\text{IR}]_t = \frac{k_{\text{kd}}(1 + a_I + a_{II} + (1 - \alpha) k_{\text{tid}})}{k_i(k_{\text{tid}} + k_{\text{tid}} + (1 - \alpha) k_{\text{tid}})}
\]

which indicates that the steady-state number of internalized ligand-
receptor complex is proportional to the ligand concentration and the production of each receptor type.

For high values of \( c \), in contrast, we obtain

\[
\frac{d[H,R]}{dt} = -\frac{k_h a_{II}}{k_h(a_h + a_{id} + (1-\alpha)k_i)} \quad \text{if } a_{II} \leq a_1, \tag{7}
\]

and

\[
\frac{d[H,R]}{dt} = -\frac{k_h a_{II}}{k_h(a_h + a_{id} + (1-\alpha)k_i)} \quad \text{if } a_{II} \leq a_2. \tag{8}
\]

Therefore, for high ligand concentration or low constitutive degradation, the steady-state number of internalized ligand-receptor complexes is controlled by the receptor with the smallest production rate and this number does not depend on the ligand concentration.

The case of high \( c \) and low constitutive degradation is especially interesting because the steady-state signal does not depend on the ligand concentration, even when the ligand is present in small quantities. An important question to address now is: can the system detect changes in concentration in this regime? When the recycling rate is much lower than the internalization rate, the number of ligand-receptor complexes in the plasma membrane equilibrates faster than all the other variables. Therefore assuming quasi-equilibrium in this variable, \( \frac{d[H,R]}{dt} = 0 \), we obtain that, upon changes in the ligand concentration (\( \Delta l \)), the changes in the number of ligand-receptor complexes in the plasma membrane (\( \Delta[H,R_{II}] \)) follow the equation

\[
0 = k_h[(l + \Delta l)[R_1] - \Delta[H,R_{II}][R_{II}] - \Delta[H,R_{II}]) - (k_{a_{II}} + k_{id} + k_i)[l[R_{II}] + \Delta[H,R_{II}]]. \tag{9}
\]

Note that we have assumed that the numbers of receptors in the plasma membrane is conserved at these time scales.

For small changes in ligand concentration, we obtain

\[
\Delta[H,R_{II}] = -\frac{k_h\Delta[l][R_1][R_{II}]}{k_h[l + R_{II}] + k_{id} + k_{id} + k_i}. \tag{10}
\]

This expression indicates that for high \( c \), low constitutive degradation, and slow recycling (compared to internalization), the system can detect changes in ligand concentration while keeping a steady-state signal that does not depend on ligand concentration.

Two-compartment model of receptor trafficking for two ligands.

The equations for a system with two ligands with concentrations \( l_1 \) and \( l_2 \) are:

\[
\frac{d[R_1]}{dt} = k_h[l_1][R_1][R_{II}] - (k_{a_{II}} + k_{id} + k_i)[l[R_1][R_{II}]]. \tag{11}
\]

\[
\frac{d[R_1]}{dt} = k_h[l_2][R_2][R_{II}] - (k_{a_{II}} + k_{id} + k_i)[l[R_2][R_{II}]]. \tag{12}
\]

\[
\frac{d[R_{II}]}{dt} = p_{II} - k_h[l_1][R_1][R_{II}] - k_h[l_2][R_2][R_{II}] - (k_{a_{II}} + k_i)[R_1] + k_{i}[R_T] + \alpha k_h[R_1][R_{II}] + \alpha k_h[R_2][R_{II}]. \tag{13}
\]

\[
\frac{d[R_{II}]}{dt} = p_{II} - k_h[l_1][R_1][R_{II}] - (k_{a_{II}} + k_i)[R_{II}] + k_{i}[R_T] + \alpha k_h[R_1][R_{II}] + \alpha k_h[R_2][R_{II}]. \tag{14}
\]

\[
\frac{d[R_{II}]}{dt} = p_{II} - k_h[l_2][R_2][R_{II}] - (k_{a_{II}} + k_i)[R_{II}] + k_{i}[R_T] + \alpha k_h[R_1][R_{II}] + \alpha k_h[R_2][R_{II}]. \tag{15}
\]

\[
\frac{d[R_1][R_{II}]}{dt} = k_h[l_1][R_1][R_{II}] - k_i[R_1][R_{II}]. \tag{16}
\]

\[
\frac{d[R_2][R_{II}]}{dt} = k_h[l_2][R_2][R_{II}] - k_i[R_2][R_{II}]. \tag{17}
\]

\[
\frac{d[R_T]}{dt} = k_i[R_1] - k_{i}[R_T]. \tag{18}
\]

\[
\frac{d[R_{II}]}{dt} = k_i[R_{II}] - k_{i}[R_{II}]. \tag{19}
\]

\[
\frac{d[R_{II}]}{dt} = k_i[R_{II}] - k_{i}[R_{II}]. \tag{20}
\]

The variables \( R_1, [R_1][R_{II}], \) and \( [R_2][R_{II}] \) are the numbers of type I and type II receptors in the plasma membrane; and \( [l[R_1][R_{II}]] \) and \( [l[R_2][R_{II}]] \) refer to the corresponding ligand-receptor complexes. The overline indicates internalized receptors and ligand-receptor complexes. The signaling activity triggered by each ligand is assumed to be proportional to the corresponding number of internalized ligand-receptor complexes. \( k_h \) is the rate constant of ligand-receptor complex formation; \( p_{II} \) and \( p_{II} \) are the rates of receptor production; \( k_i, k_{id}, k_{id}, \) and \( k_{id} \) are the internalization, recycling, constitutive degradation, and ligand-induced degradation rate constants; and \( \alpha \) is the fraction of active receptors that are recycled back to the plasma membrane and can interact again with the ligand.

Coupled signaling and perfect adaptation. Computational modeling offers precise insights into the functioning of the TGF-β pathway. It is possible to go a step further and generalize the conditions that give rise to different qualitative types of behavior.

Let us consider two ligands (\( l_1 \) and \( l_2 \), one type I receptor (\( R_1 \)), and two completely II receptors (\( R_{II} \) and \( R_{II} \)). The type I receptor is shared among the two ligand-receptor complexes (\( l_1[R_1][R_{II}] \) and \( l_2[R_1][R_{II}] \)).

The following conservation equations refer to the common type I receptor at steady-state.

Under stationary conditions, the number of receptors produced (by gene expression) is equal to the number of receptors degraded:

\[
p = d_{const} + d_{grad}. \tag{21}
\]

where \( p \) is the receptor production rate; and \( d_{const} \) and \( d_{grad} \) are the constitutive and ligand-induced degradation rates, respectively. Assuming that a fraction \( \delta \) of the activated receptors is degraded through a ligand-induced degradation process, we can express \( d_{grad} \) as

\[
d_{grad} = \delta(l_{1a} + l_{2a}) \quad \text{with } \delta \in (0,1). \tag{22}
\]

where \( l_{1a} \) and \( l_{2a} \) are the rates of formation of the ligand-receptor complexes (\( l_1[R_1][R_{II}] \) and \( l_2[R_1][R_{II}] \), respectively). Therefore, \( p = d_{const} + \delta(l_{1a} + l_{2a}) \).

We explicitly consider the following two limiting cases:

In the first case, there is no ligand-induced degradation (\( d_{const} = 0, \delta = 1 \)). Therefore, we have \( d_{grad} = 0 \), which leads to \( p = d_{const}. \) Because \( d_{const} = d_{const}(R_1) \) is a function of the total number of receptors \( R_1 \), the previous condition indicates that the number of receptors remains constant among the two ligand-receptor complexes (\( [l_1[R_1][R_{II}]] = [l_2[R_1][R_{II}]] \)). Under these conditions, if the constitutive receptor degradation follows first order kinetics, \( d_{const} = \gamma R_1 \), then \( R_1 = R_{1o} / p_{II} \).

Under these conditions, the completely coupled mode of signal integration arises even for low ligand concentrations.

The conditions that lead to the completely coupled mode of signal integration also lead to perfect adaptation for a single ligand. Consider, for example, \( l_{2a} = 0 \). When there is only ligand-induced degradation, the fact that the rate of formation of complexes remains constant implies \( p = \delta l_{1a} \). The steady state of the system is fixed irrespective of the ligand concentration. Consequently, changes in ligand concentration can only elicit transient responses that completely adapt to the prestimulus level and the system exhibits perfect adaptation \([29]\).

Acknowledgments

We are indebted to Joan Massagué and Van Le for invaluable help during the early stages of this work. We are also very grateful to Gary Bader, Debora Marks, Leonor Saiz, Nikolaus Schultz, Wenying Shou, and Stan Shulvans for numerous discussions, comments, and suggestions. CS was funded in part by the Alfred W. Bresler Scholars Endowment Fund.

Author contributions. JMGV conceived and designed the experiments, performed the experiments, and analyzed the data. RJ and CS contributed reagents/materials/analysis tools. JMGV and RJ wrote the paper. CS critically edited the manuscript.

Competing interests. The authors have declared that no competing interests exist.
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