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A mechanism for Src kinase-dependent signaling by non-catalytic receptors

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Abstract

A fundamental issue in cell biology is how signals are transmitted across membranes. A variety of transmembrane receptors, including multichain immune recognition receptors, lack catalytic activity and require Src family kinases (SFKs) for signal transduction. However, many receptors only bind and activate SFKs after ligand-induced receptor dimerization. This presents a conundrum: How do SFKs sense the dimerization of receptors to which they are not already bound? Most proposals to resolve this enigma invoke additional players, such as lipid rafts or receptor conformational changes. Here we used simple thermodynamics to show that SFK activation is a natural outcome of clustering of receptors with SFK phosphorylation sites, provided that there is phosphorylation-dependent receptor-SFK association and an SFK bound to one receptor can phosphorylate the second receptor or its associated SFK in a dimer. A simple system of receptor, SFK and an unregulated protein tyrosine phosphatase (PTP) can account for ligand-induced changes in phosphorylation observed in cells. We suggest that a core signaling system comprising a receptor with SFK phosphorylation sites, an SFK and an unregulated PTP provides a robust mechanism for transmembrane signal transduction. Other events that regulate signaling in specific cases may have evolved for fine-tuning of this basic mechanism.

Keywords

Ephrin; C-type lectin; Nephrin; CDCP1; Reelin; Dab1; Multichain immune recognition receptors; T cell receptor; B cell receptor; FcεRI

Cells detect their environment through a wide variety of receptors. While signaling via receptors with intracellular catalytic activities or channel functions is well understood, signaling by a variety of receptors that are linked to Src family kinases (SFKs) remains unresolved (1–11). SFKs are a subgroup of the non-receptor tyrosine kinases. They are allosteric enzymes with at least two conformational states that are differentially stabilized by protein-protein interactions and by phosphorylation-dephosphorylation at two different sites (12–14). Active SFKs can bind to other proteins through a phosphorylation-independent SH3 domain and a phosphorylation-dependent SH2 domain. This allows for stabilization of the active state.

Some receptors, such as CD4, are constitutively bound to an SFK (15,16). Clustering of CD4 by its ligand brings the bound SFKs together and allows for a stimulating intermolecular phosphorylation event (17). This mechanism is comparable to that established for receptor

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tyrosine kinases (RTKs)(Fig. 1b) (18,19). RTK kinase domains have a low basal activity, which is effectively opposed by cellular protein tyrosine phosphatases (PTPs). When the kinase domains are brought together they undergo intermolecular phosphorylation, which stabilizes a new conformation with increased activity.

Unlike CD4, however, many other SFK-dependent receptors show little SFK binding prior to clustering, so the RTK model does not apply. Some such receptors are listed in Table1. They include immune regulatory receptors and several cell-matrix and cell-cell signaling receptors (1–11). Here we call these receptors SDRs (SFK-dependent receptors). SDR signaling is initiated by clustering (dimerization or oligomerization) of the receptors by ligand (1–11). This induces SDR tyrosine phosphorylation, SFK activation and formation of phosphorylation-dependent SFK-SDR complexes via the SFK SH2 domain.

Various mechanisms have been proposed to explain how dimerization of an SDR is communicated to an SFK that is not previously bound. For example, dimerization of the T cell receptor (TCR) may alter the conformation of its cytoplasmic domain so that it partitions into lipid rafts, where it is exposed to raft-associated SFKs (20–23). Alternatively, antigen-presenting cells may displace transmembrane PTPs with bulky extracellular domains from the vicinity of TCRs (24). Or, an SFK inhibitor, Csk, may be lost from the vicinity of the TCR due to rapid dephosphorylation of its membrane anchor, Cbp/PAG (25). However, none of these mechanisms is straightforward. Lipid rafts provide an environment that may inhibit SFKs (26), they are more important for maintaining than for initiating TCR signaling (27) and they do not coincide with initial SFK-dependent clusters of TCRs (28). Physical displacement of transmembrane PTPs seems unlikely for receptors with small ligands. And in myeloid cells Cbp/PAG phosphorylation increases during the initial activation of FcεRI, which would seem to oppose SFK activation (25). Furthermore, none of these mechanisms satisfactorily explains a recent observation (29). The Dab1 component of the Reelin receptor-Dab1 complex contains SFK sites that are phosphorylated when the Reelin receptors are clustered. This phosphorylation is also induced when the Dab1 component is artificially dimerized in the cytosol (29). This suggests that activation can occur by clustering phosphorylation sites in the absence of membranes or apparent mechanisms for regulating kinases or phosphatases.

Here we have explored the properties of a minimal signaling system. We asked what happens when a hypothetical SDR changes from monomer to dimer state. We assumed that the only association between SDR and SFK requires phosphorylated SDR, and there are no conformation changes or compartmentalization of the receptor and no external influences on SFK or PTP activity. Using first principles, we found that that mass action can explain significant increases in SDR phosphorylation and SFK activation following SDR dimerization.

RESULTS

The model is shown in Fig 1c. In the absence of ligand, basal activity of the SFK (*E*) causes low-level phosphorylation of the monomeric receptor (*R*) to create *R** (where asterisk represents phosphate). The SH2 domain of *E* can associate with *R** to form *R*-E*, protecting *R** from dephosphorylation (30). However, association-dissociation is rapid (31), and constant PTP activity keeps the phosphorylation level low. In the presence of ligand, the receptor is a dimer (*RR*). Now, phosphorylation of one receptor molecule creates *RR** and allows binding of *E* to create *RR*-E*. The second receptor in the dimer can now be phosphorylated in an essentially "intra-molecular" reaction (32,33). This is a key step, and creates *R*R*-E* to which a second *E* may bind, creating *E-R*R*-E*. In the second key step, the two SFKs in this tetramer can undergo intermolecular phosphorylation and become more active (14,34–36). This creates the highly active *E*-R*R*-E** Subsequent dissociation of ligand will allow release of *E**,

raising the activity of *E* in the cell and stimulating further phosphorylation of *RR*. With time, the system returns to baseline by PTP action.

Even though the model contains only SDR, SFK and PTP, it is more complex than the RTK model, and *a priori* seemed unlikely to explain the experimentally-observed increases in SDR phosphorylation or SFK activation after ligand-induced receptor dimerization. We therefore tested the model by analyzing the component reactions and applying the laws of mass action.

We first analyzed the effect of SDR-SFK association on SDR phosphorylation (Appendices 1 and 2; all Appendices are available as Supporting Information). We then analyzed the effect of SDR-SFK association on SFK phosphorylation (Appendix 3). This breakdown allowed us to solve the simplest kinetic model representing each effect algebraically, without resort to complex simulation or numerical methods. The Supporting Information includes Excel spreadsheets that will calculate results for any chosen values of input parameters, and selected results are plotted in Fig. 2– Fig. 4.

1. Positive and negative feedback effects due to SFK binding to monomeric receptors

We analyzed the effects of SFK (*E*) binding to phosphorylated monomeric receptors (*R**) (Fig. 2a). SFKs are allosteric enzymes with at least two conformational states (12–14): closed, inactive $E^{\dagger}$ and open, active *E* (Fig. 1a; *E* can be further activated by phosphorylation in the activation loop, but for the present we consider only *E* in its low activity state). The relative amount of $E^{\dagger}$ (the ratio *Q*) is regulated by C-terminal phosphorylation at a residue that stabilizes the $E^{\dagger}$ state. We are not proposing any regulation of phosphorylation of the C-terminal site, so we assume that *Q* does not change when receptors are dimerized. The active *E* phosphorylates *R* with a bimolecular rate constant k_1, and binds *R** with association constant K_3. The PTP is in excess and not regulated, so appears as a pseudo first order rate constant, k_2, for dephosphorylating *R**.

We calculated the fractional phosphorylation of receptors, f_R, under different conditions of SFK and PTP activity (Appendix 1). f_R is commonly measured experimentally, for example, by Western blotting, and represents the amount of *R** molecules relative to the total (Fig. 2a). The cellular environment was represented by the ratio of SFK and PTP activities, θ_R (Fig. 2a). Note that the cellular environment does not change when receptors dimerize, but will differ according to cell type and conditions.

The best way to understand the effect of binding of *E* to *R** is to compare it with the simplest situation of no binding. In this case, we have the standard “hyperbolic response curve” with 50% phosphorylation ($f_R = 0.5$) when the rates of phosphorylation and dephosphorylation are balanced ($\theta_R = 1$). Increasing or decreasing the ratio of kinase to phosphatase activities increases or decreases f_R accordingly (Fig. 2b, red line). Binding of *E* to *R** has two effects: First, it reduces the amount of free *E* able to phosphorylate more *R*. This is a negative feedback or sequestration effect (37). In addition, the *R**-*E* complexes are protected from dephosphorylation (30), so this stabilizes *R** and causes a positive feedback. These two counteracting influences mean that more or less *E* activity may be needed for 50% phosphorylation - the response curve is shifted left by positive feedback and right by negative feedback (Fig. 2b, + and - red arrows, respectively). Fig. 2b (black lines) shows the effect of changing the association constant of *E* for *R** (K_3, Fig. 2a). At very low binding (Fig. 2b, x's), the curve overlays the control (red line), but with high binding (diamonds), the response curve is shifted far to the left. The positive feedback effect predominates. On the other hand, reducing the total concentration of *E* relative to *R*, or raising *Q*, which allows for a reservoir of closed-conformation, inactive $E^{\dagger}$, causes negative feedback to predominate and shifts the curve to the right (Fig 2b, blue lines).

2. Effect of trans-phosphorylation in dimeric receptor complexes

The essence of our model for SDR mediated signaling is an additional positive feedback pathway for a dimeric receptor: an "intramolecular" phosphorylation of the *RR*-E* complex to form *R*R*-E* (32,33).. *RR* of course can still be phosphorylated on the two subunits independently by the bimolecular reaction, and phosphorylated subunit(s) can bind to *E* (Fig. 3a). This receptor trans-phosphorylation effect is quantified by parameter σ, which includes the association constant K_3 and the uni-molecular trans-phosphorylation constant, k_4 (Fig. 3a). When *R* is a monomer, σ = 0 (Fig. 3b, red line). However, when *R* is a dimer, σ has some positive value and the value of f_R is increased (Fig 3b, black lines; Appendix 2). Thus, for given θ_R, receptor dimerization increases f_R, moving vertically on Fig 3b from the red line to the appropriate black line depending on the value of σ for the dimer (red arrow). In the example shown, dimerization of receptors with σ = 1000 causes a ~15-fold increase in phosphorylation at θ_R = 0.05 (Fig. 3c, red arrow), but a smaller change at either higher or lower values of θ_R. The importance of θ_R fits with biological expectations: increasing the PTP activity too high will prevent receptor activation even at high levels of extracellular stimulus, and decreasing the PTP too low will result in activation even in the absence of stimulus. The concentration and activity of PTP set a threshold and appropriate range for receptor activation.

Surprisingly, we found that receptor monomers and dimers are similarly (but not identically) affected by negative and positive feedback in Sec. 1 (Appendix 2, Fig S1). We calculated receptor phosphorylation for a variety of parameter values that shift the monomer response to left or right (Fig. 2b), and found that they similarly affect the dimer, so the set of curves shown in Fig 3b shifts left or right along the abscissa (Fig. S1). Therefore, the effect of trans-phosphorylation in the *RR*-E* complex (Fig. 3b) is similar over a wide range of starting concentrations and other constants, such as *Q* (the fraction of *E* in the inactive conformation). A surprising result is that even if all the SFK is in the active conformation (*Q* = 0, e.g., in cells expressing a mutationally-activated allele, or in cells where the C-terminal tyrosine of the SFK is not phosphorylated), dimerization of receptors can still cause a considerable increase in phosphorylation (Fig. S1). The effects of feedback on receptor monomers and dimers are not identical however, so changes in K_3, R_t, E_t or *Q* do have small effects on the fold increase in phosphorylation induced by receptor dimerization.

3. Effect of SFK trans-phosphorylation

We next analyzed the effect of SFK phosphorylation in its activation loop. The open, low activity form of SFK (*E*) can be activated ϕ-fold by intermolecular phosphorylation (14,34–36), with bimolecular rate constant q_1 (Fig. 4a). *E* bound to various forms of *R** can also be phosphorylated by *E*. All forms of *E**, whether free or bound, are dephosphorylated with pseudo-first order rate constant q_2. All other binding and phosphorylation reactions are as before.

We introduce a control parameter for SFK phosphorylation (θ_E), which represents the balance between bi-molecular phosphorylation of *E* and its dephosphorylation by PTP. Like θ_R, θ_E is the same for monomer and dimer receptors. It sets the tone for the system, and can vary in different cell types. For receptor monomers, where *E* influences *R* phosphorylation but not vice versa, the fraction of *E* that is phosphorylated, f_E, depends only on θ_E. The relationship between θ_E and f_E is the same as between θ_R and f_R (eg. see red line in Fig. 2b). Note that the only role of *Q*, the equilibrium between inactive and active conformations, is to change θ_E (Appendix 3, Eqns. 1 and Eqn. 4). This means that the following argument applies equally to cells in which the SFK is or is not phosphorylated at its C terminal tyrosine residue.

With receptor dimers, doubly-phosphorylated *R*R** can act as a scaffold for forming *E-R*R*-E* complexes, and phosphorylation and activation of *E* in these complexes becomes uni-

molecular (rate constant q_3), and is greatly increased. This SFK trans-phosphorylation effect is represented by ξ. When receptors are monomers, there is no trans-phosphorylation, ξ = 0, and f_E depends on θ_E but not θ_R (Fig. 4b, red line). However, with receptor dimers, ξ is a positive number, and f_E now depends on θ_E, ξ, and on the concentration of *R*R**. This latter depends on f_R, which in turn depends on θ_R, θ_E, ξ and ϕ (*E** activity relative to *E*). This circular relationship between phosphorylation of *E* (f_E) and *R* (f_R) represents positive feedback, in which increased *R* phosphorylation leads to increased *R*R**, increased scaffolding effect, increased *E** and increased *E* activity to phosphorylate *R*.

The fraction of *E* phosphorylation (f_E) is graphed relative to θ_R for given θ_E and ϕ and various ξ in Fig. 4b (blue lines). Receptor dimerization at given θ_R increases *E* phosphorylation vertically from the horizontal red line to the appropriate blue line, dependent on ξ(red arrow). The fraction of phosphorylated receptors, f_R, also increases, moving vertically on Fig 4c from the red line to the appropriate black line depending on the value of ξ for the dimer (red arrow). In the example shown, with ξ = 100, ϕ = 10, θ_E = 0.1 and θ_R = 0.1, we find that f_E increases ~7-fold and f_R increases ~2.7-fold (Fig. 4b and d, squares). Depending on starting conditions, the increases in SFK and receptor phosphorylation due to SFK trans-phosphorylation can be quite large (Fig. S2). Note that we ignored the receptor trans-phosphorylation effect (Fig. 3) in these calculations. Receptor trans-phosphorylation would further increase f_R, which would further increase f_E, which would further increase f_R.

4. Values for the parameters from experimental measurements

Values for receptor trans-phosphorylation (σ), SFK trans-phosphorylation (ξ), SFK activation by phosphorylation (ϕ) and total cellular concentrations of SFK (E_t) and receptor (R_t) were obtained from the literature (Table 2, available in Supporting Information). Each of these values may range over several orders of magnitude: σ from 20 to 5000, ξ from 2 to 800, and ϕ from 4 to 20. As explained above, the parameter *Q*, the relative amount of SFK in the inactive conformation, has approximately the same effect on phosphorylation of receptor monomers as it does on receptor dimers. To estimate the control parameters for receptor and SFK phosphorylation, θ_R and θ_E, we made use of the observation that low levels of phosphorylated receptors and SFKs are detectable in unstimulated cells (38–40), suggesting that these quantities are in the range of ~0.05–0.1. The preceding values were used for Fig. 2–Fig. 4. The results show that receptor and SFK trans-phosphorylation effects both contribute to ligand-induced phosphorylation of SDRs, and if both effects occur simultaneously, with the expected mutual reinforcement, then even greater increases are expected. In addition, further increases are predicted if receptors cluster into higher-order oligomers.

DISCUSSION

We analyzed a model for signal transduction by SFK-dependent receptors (Fig 1c). We found that dimerization significantly stimulates receptor and SFK phosphorylation without requiring any other regulatory events. Importantly, the model does not require regulation of SFK activity either by C-terminal phosphorylation/dephosphorylation or by stabilization of the active conformation of the SFK when bound to phosphorylated receptor. The results are surprising because there are many more steps than for the RTK model (Fig. 1b), and many of the steps are inefficient. For example, only a subset of SFK molecules are in a conformation that can bind receptors; phosphorylation of SFKs in the activation loop causes only a modest activation; the scaffolding effects require ternary and quaternary protein complexes; and the cellular concentrations of phosphorylated receptors and SFKs are low relative to their mutual binding affinity. Nevertheless, receptor dimerization will strongly increase SDR phosphorylation and SFK activation, provided that the values for receptor and SFK concentrations from cells and kinetic constants from in vitro assays and certain other assumptions (see below) are accurate.

The ability of the model to predict effects that are consistent with biological measurements, without recourse to detailed computational modeling, suggests that the minimal system may underlie biological reality.

We had initially expected that the greatest extent of dimerization-induced stimulation would be two-fold, with one bound SFK phosphorylating two receptor molecules instead of one. However, much greater than two-fold increases may result, depending on cellular conditions (Fig. 3c, 4b, 4d). The "something for nothing" effect does not come at no expense, but is driven by "futile" cycles of phosphorylation-dephosphorylation. Another result of the analysis is that the signaling is stimulated in direct proportion to the fraction of receptors that are dimerized. There is no element of a "switch like" (cooperative or ultra-sensitive) response. However, the same applies to RTKs, where binding of a ligand to induce dimerization of a pair of RTKs activates only that one pair. Despite this initially linear response, positive and negative feedback events downstream of the initial receptor activation can allow a switch-like cellular outcome. In the case of multichain immune recognition receptors (MIRRs), this is called kinetic proofreading or serial engagement (41).

The validity of the results relies on the validity of the underlying assumptions. To our knowledge, the assumptions are reasonable and supported by the literature. The first is that molecular flexibility allows an SFK bound to one receptor in a dimer to phosphorylate its partner. It is known that an SFK bound to one phosphotyrosine can phosphorylate other tyrosine residues in the same substrate (42,43), and others have proposed trans-phosphorylation of clustered SDRs by associated SFKs (32,33). Activated SFKs have few inter-domain contacts (12,44), and molecular dynamics simulations predict flexibility (45,46). We also suspect that SDRs are flexible, based on their lack of predicted secondary structure elements and the experimental finding that TCR proximity but not orientation is critical for activation (47). Thus it seems likely that receptor trans-phosphorylation can occur.

We also assume that SFK phosphorylation in the activation loop is intermolecular and can occur on or off membranes. This is supported by experiment (14,34–36). Phosphorylation in solution is inefficient because the SFK concentration is too low (35,36). However, SFKs are more likely to trans-phosphorylate when bound to dimeric receptors. Indeed, SFKs that are associated with active SDRs have more phosphotyrosine than unbound SFKs (48).

The model does not require regulation of PTPs. They are passive players that keep the phosphorylation of monomeric receptors and SFKs low and reset the system when ligand is removed. They are also important because they cannot dephosphorylate sites to which other proteins are bound (30), allowing a positive feedback effect on phosphorylated receptors. There is abundant evidence that the balance of PTP to SFK is critical for MIRR signaling (49). Although we do not require the PTP to be regulated, if it is, e.g. by displacement from dimerized SDRs, by reactive oxygen produced by active SFKs, or by active recruitment to phosphorylated SDRs (24,26,50), then receptor activation may be further increased.

SUMMARY AND CONCLUSIONS

We have found that dimerization can induce increasing receptor phosphorylation by SFK, and SFK activation, by mass action kinetics alone. The increasing phosphorylation upon dimerization is due to a positive feedback between receptor phosphorylation, SFK binding, and SFK activation by phosphorylation in the kinase domain. The same principles can also explain cooperative phosphorylation of proteins with multiple phosphorylation sites. They also suggest that other SH2 domain-containing tyrosine kinases may be similarly regulated, since the SFK SH3 domain and C-terminal phosphate are not involved. However, we wish to emphasize that we do not exclude roles for many other mechanisms in the system, such as

changes in PTP activity, the lipid environment or kinases or PTPs that act on SFKs, but the results suggest that such changes are not necessary and may be evolutionary fine-tuning of a basically simple system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Abbreviations

FcεRI
: immunoglobulin Fc receptor type εRI

PTP
: protein tyrosine phosphatase

SDR
: SFK-dependent receptor

SFK
: Src family kinase

SH2
: Src homology 2

SH3
: Src homology 3

RTK
: receptor tyrosine kinase

TCR
: T cell receptor

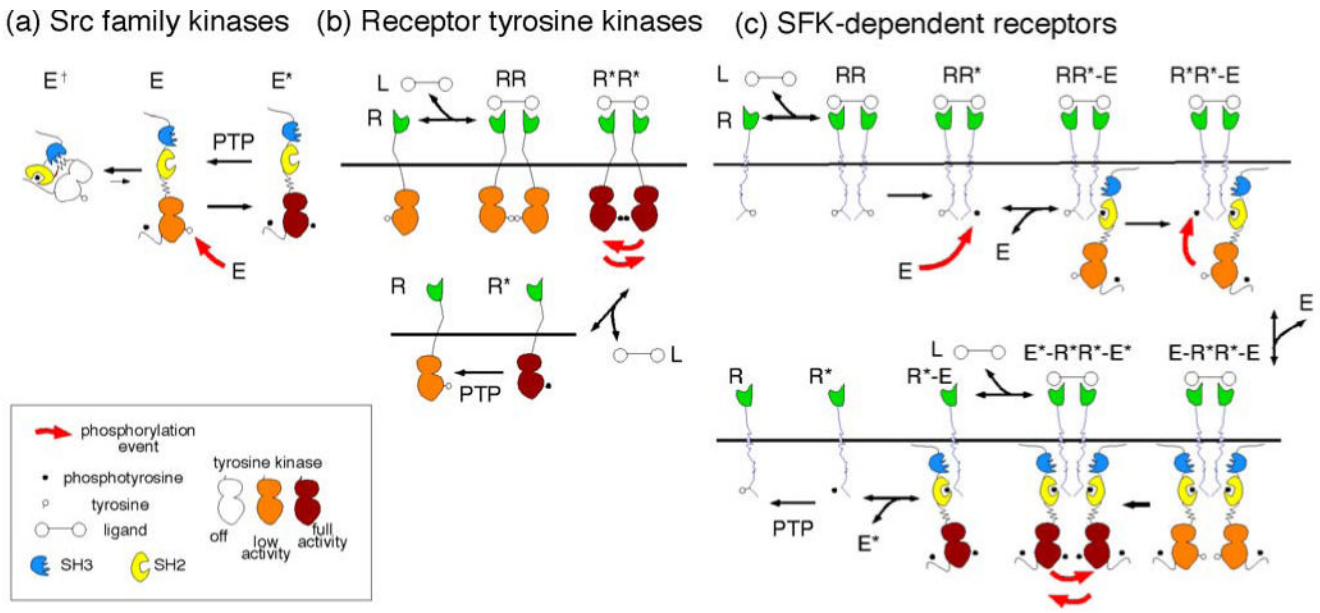

FIGURE 1. Models for regulation of receptor tyrosine kinases and SFK-dependent non-catalytic receptors

(a) Structure and regulation of SFKs. SFKs have 3 domains: SH3 (blue, phosphorylation-independent binding, not considered here), SH2 (yellow, phosphorylation-dependent binding) and kinase (white, orange or crimson according to increasing activity). The SFK can adopt two main conformation states: "closed" inactive ($E^{\dagger}$) and "open" low (E) and high (E^*) activity forms. E is converted to E^* by intermolecular phosphorylation in the activation loop. In addition, phosphorylation at the C terminus alters the balance between the closed and open conformations by stabilizing the closed form. This phosphorylation is not regulated in the model, so is not considered further.

(b) Regulation of RTKs. The kinase domains of monomeric RTKs, R, are inhibited by intramolecular interactions, and have low basal activity (orange) which is readily reversed by PTPs. Following dimerization by ligand, L, intermolecular phosphorylation of the activation loop occurs, and the kinase is activated (crimson). If ligand dissociates, monomeric receptors will remain in the phosphorylated, active state until dephosphorylated by PTPs. This allows for hysteresis in signaling.

(c) Proposed model for regulation of SDRs. Only a small fraction of monomeric SDRs (R) are phosphorylated. After dimerization by ligand, this phosphorylation may allow binding of an SFK (RR^*-E complex). This may lead to an "intramolecular" phosphorylation to form a R^*R^*-E complex. This stimulates receptor phosphorylation. If a second E binds, to form an E-R^*R^*-E complex, then intermolecular phosphorylation of E will be stimulated, and the E^*-R^*R^*-E^* complex will have high kinase activity. Phosphorylated E^* may be released into the cytosol, to phosphorylate more receptors. This would provide a mechanism for hysteresis in signaling.

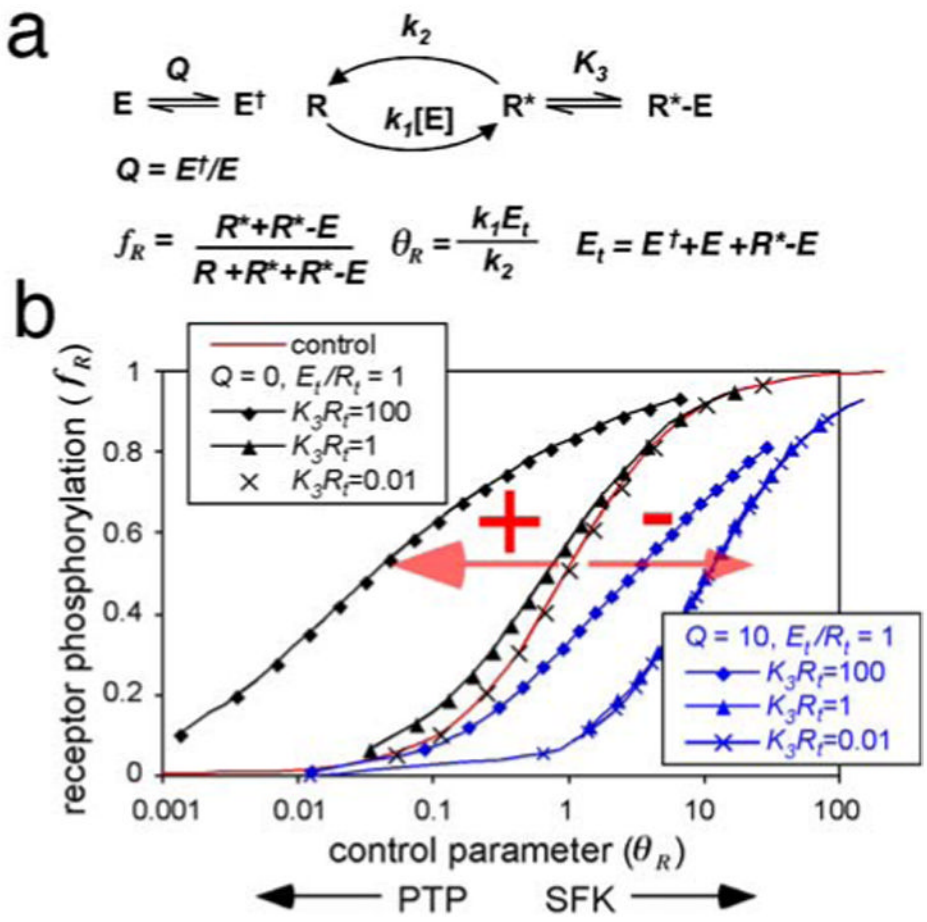

FIGURE 2. Positive and negative feedback effects due to SFK binding to monomeric receptors

(a) Reactions and equations describing the conformation states of the SFK and monomeric receptor phosphorylation. These reactions are solved in Appendix 1.

(b) Fractional receptor phosphorylation (f_R) as a function of the ratio of SFK and PTP activities (the control parameter for receptor phosphorylation, θ_R). Results are shown for different values of the equilibrium between closed and open SFK (Q), the ratio of total SFK to receptor (E_t/R_t), and the product of receptor concentration and binding affinity (K_3R_t). The control (red line) is for no binding of E to R^*. Black lines show results where positive feedback (+) predominates. Blue lines show where negative feedback predominates (−).

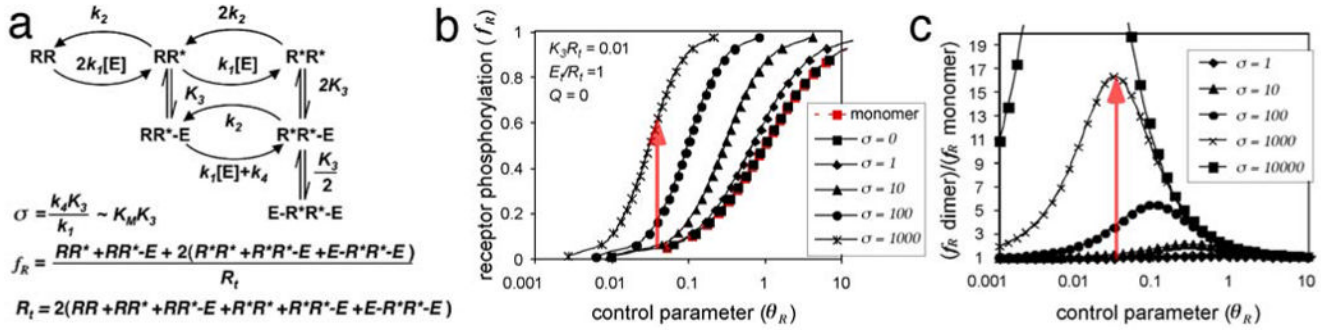

FIGURE 3. Trans-phosphorylation of dimeric receptors

(a) Reactions and equations describing the interactions of open-conformation *E* with dimeric receptor *RR*. The phosphorylation/dephosphorylation and association constants are as in Fig. 2, except k_4 represents receptor trans-phosphorylation within a *RR**-*E* complex. These reactions are solved in Appendix 2.

(b) Fractional receptor phosphorylation (f_R) as a function of the control parameter for receptor phosphorylation (θ_R), for particular values of Q, E_t/R_t and K_3R_t. The control (red) is for monomeric receptors. Black lines show results for dimeric receptors with various values of the receptor trans-phosphorylation parameter (σ). Dimerization does not change any parameter except σ, which increases f_R (red arrow).

(c) Relative increase in receptor phosphorylation due to dimerization.

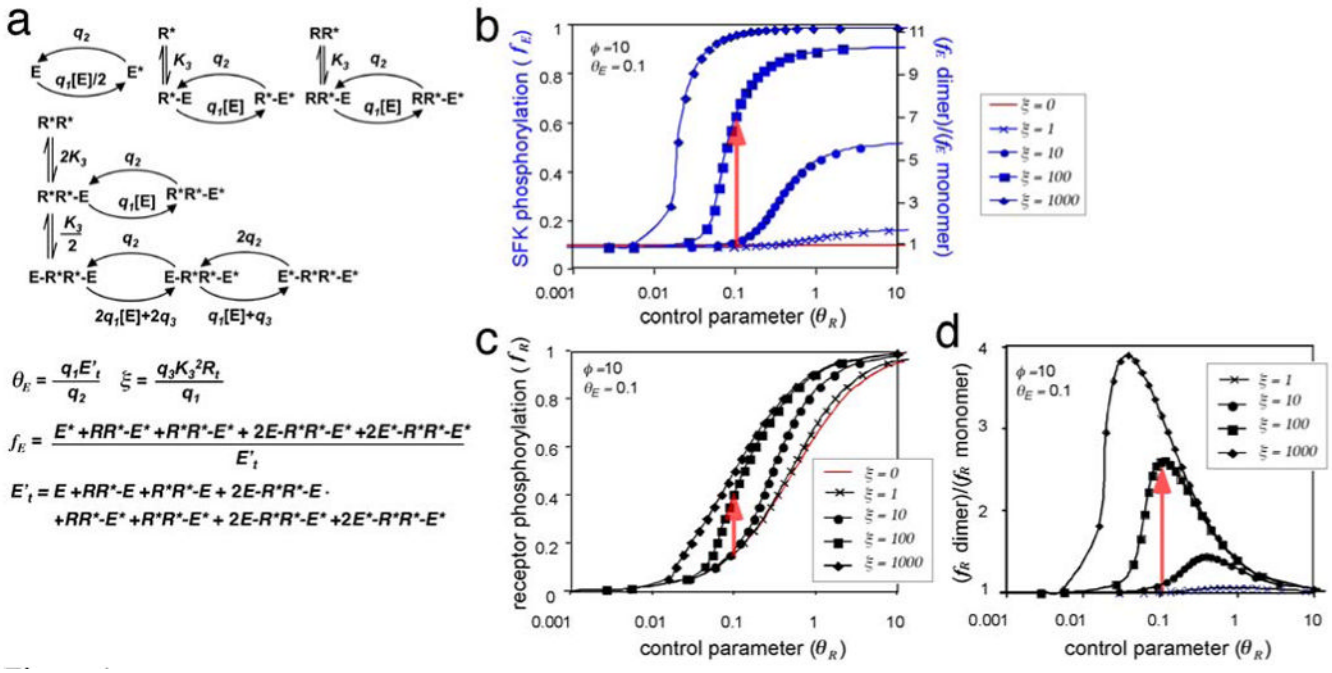

FIGURE 4. Trans-phosphorylation of SFK

(a) Reactions and equations describing the intermolecular phosphorylation of *E*. Phosphorylation of *E*, alone or complexed with various forms of *R**, occurs with bi-molecular rate constant q_1, and dephosphorylation occurs with pseudo-first order rate constant q_2. q_4 represents SFK trans-phosphorylation within a *E*-*R***R**-*E* complex. These reactions are solved in Appendix 3.

(b) Fractional SFK phosphorylation (f_E) as a function of the control parameter for receptor phosphorylation (θ_R), for particular values of the phosphorylation-induced increase in SFK activity (ϕ) and the control parameter for SFK phosphorylation (θ_E). The control (red) is for monomeric receptors. Blue lines show results for dimeric receptors with various values of the SFK trans-phosphorylation parameter (ξ). Dimerization does not change any parameter except σ, which increases f_E (red arrow).

(c) Fractional receptor phosphorylation. Same parameters as in (b).

(d) Relative increase in receptor phosphorylation due to dimerization. These graphs were constructed by interpolation of the data in (c).

Table 1

SFK-Dependent Receptors (SDRs)

SDR	SFK phosphorylation site(s) [a]	References
Multichain immune recognition receptors, e.g., T cell receptor ξ chain, B cell receptor Igα and β chains, FcεRIγ	YXXL/I(6–8 [b])YXXL/I	(1–3)
Inhibitory immune receptors, e.g., FcεRIIB, SHPS, Siglecs	(I/V/L/S)xYxx(L/V)	(4)
Lectins CLEC-2 and Dectin-1	DEDGYXXL	(5)
Ephrin B family	YXXV(3)YXXPXYXXQ(10)YYKV	(6)
Nephrin [c]	Five repeats of YDXV	(7)
CDCP1	YXXI(5)YXXL	(8)
Myelin-associated glycoprotein	YAEI	(9)
Reelin receptor-Dab1 complexes	YQXI(9)YQXI	(10,11)

[a] Y, confirmed SFK phosphorylation site; Y, phosphorylation not shown.

[b] Numbers in parentheses indicate spacing between sites.

[c] Nephrin also binds to SFK SH3 domains.