The Complexity of Complexes in Signal Transduction

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Abstract: Many activities of cells are controlled by cell-surface receptors, which in response to ligands, trigger intracellular signaling reactions that elicit cellular responses. A hallmark of these signaling reactions is the reversible nucleation of multicomponent complexes, which typically begin to assemble when ligand-receptor binding allows an enzyme, often a kinase, to create docking sites for signaling molecules through chemical modifications, such as tyrosine phosphorylation. One function of such docking sites is the co-localization of enzymes with their substrates, which can enhance both enzyme activity and specificity. The directed assembly of complexes can also influence the sensitivity of cellular responses to ligand-receptor binding kinetics and determine whether a cellular response is up- or downregulated in response to a ligand stimulus. The full functional implications of ligand-stimulated complex formation are difficult to discern intuitively. Complex formation is governed by conditional interactions among multivalent signaling molecules and influenced by quantitative properties of both the components in a system and the system itself. Even a simple list of the complexes that can potentially form in response to a ligand stimulus is problematic because of the number of ways signaling molecules can be modified and combined. Here, we review the role of multicomponent complexes in signal transduction and advocate the use of mathematical models that incorporate detail at the level of molecular domains to study this important aspect of cellular signaling.

Keywords: multivalent binding; regulated recruitment; receptor; signal transduction; mathematical model

INTRODUCTION

A cell uses cell-surface receptors to constantly monitor its environment and initiate responses to environmental cues, e.g., signals such as growth factors and cytokines. The range of signals, typically ligands, a cell can detect and the concentrations at which ligands can be detected are determined by the array of receptors on the cell's surface. When a receptor encounters an agonist ligand, ligand-receptor interaction triggers a cascade of intracellular signaling reactions that can lead to a variety of cellular responses, such as the secretion of mediators of cell–cell communication, changes in gene expression, and cell proliferation. For an overview of cellular signaling, see Hunter (2000). Because receptor-mediated signal transduction plays a central role in regulating a panoply of cellular activities, improved understanding of receptor signaling has a number of potential practical applications, from the rational design of drugs and vaccines to the engineering of cells for biotechnological purposes.

So far, much of the effort to understand receptor-mediated signal transduction has been aimed at identifying the molecules that participate in specific signaling cascades and at qualitatively characterizing the activities and interactions of these molecules. Thus, for a well-studied system, we might have a list of parts and enzymatic activities, knowledge of where each molecule acts in the signaling cascade (e.g., the activity of molecule A is required for the activity of molecule B), and knowledge of protein–protein interactions at the domain level (e.g., molecule A interacts with molecule B via binding of domain X in A to domain Y in B). Here, we use ''domain'' as a general term for a functional component of a protein, such as an individual amino acid residue, like a tyrosine that is phosphorylated, a short motif or segment of a polypeptide chain that is recognized by a binding partner, like the immunoreceptor tyrosine-based activation motif (ITAM) (Cambier, 1995), a large modular segment of a polypeptide chain with binding or catalytic activity, like the kinase domain of a receptor tyrosine kinase (Schlessinger, 2000), or a subunit of a multimeric protein, like the α chain of FcεRI (Kinet, 1999).

The acquisition of qualitative information about the cellular signaling apparatus is no small task, in part because the typical protein involved in a signaling cascade is a complex machine subject to multiple layers of regulation. However, the ultimate goal of studying signal transduction is to understand how the components in a signaling cascade work together as a system to direct cellular responses to changes in the extracellular environment. This level of understanding will require quantitative characterization of

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signaling components and their interactions (e.g., measurement of concentrations and rate constants) and will be achieved when we are able to accurately predict how a cell responds to an array of external signals over a range of intracellular operating conditions. Mathematical models provide the framework for achieving such a predictive systems-level understanding.

Appropriately, mathematical modeling of signal transduction is now emerging as a prominent field of research in systems biology (Bhalla and Iyengar, 1999; Endy and Brent, 2001; Kitano, 2002; Taussig et al., 2002; Wiley et al., 2003). Here, we present our perspective on modeling of signal transduction, advocating models that track the interactions, modifications, and activities of molecular domains, the fundamental elements of signal transduction systems (Pawson and Nash, 2003). We will focus on an omnipresent feature of signal transduction that we feel presents a major challenge to modelers, the assembly of multicomponent complexes through conditional multivalent binding. We speak of conditional multivalent binding, because the activity of a molecular domain in a binding reaction can be modulated, for example, by phosphorylation, and a signaling molecule typically contains multiple domains, which mediate interactions with multiple molecules. A consequence of conditional multivalent interactions among signaling molecules is the possibility that a ligand stimulus will induce the formation of a number of chemically distinct multicomponent complexes during the process of signal transduction. We will discuss the significant but manageable challenges that ligand-induced assembly of diverse complexes pose for the development of models, how these challenges have been addressed, and how they might be dealt with in the future. We will also discuss how complex assembly can have surprising functional consequences and comment on recent studies (Faeder et al., 2003; Goldstein et al., 2002; Hlavacek et al., 2001, 2002) that show how the behavior of a signal transduction system can depend qualitatively and nonlinearly on quantitative factors, such as the relative abundance of a signaling molecule or competition between concurrent processes that have counteracting effects.

MULTICOMPONENT COMPLEXES

Much of receptor signaling consists of construction projects that take place just below the cell surface. In Figure 1, for each of four systems, we have schematically illustrated one of the multicomponent membrane-proximal complexes that can form as a result of ligand-receptor binding. The mechanisms responsible for ligand-induced nucleation of such intracellular complexes are similar for a wide variety of systems. Typically, ligand-receptor binding allows an enzyme to create binding sites around which complexes nucleate. Below, we discuss the specific processes directed by the epidermal growth factor receptor (EGFR) and the high-affinity receptor for IgE antibody (FcεRI) that lead to the formation of multicomponent intracellular complexes

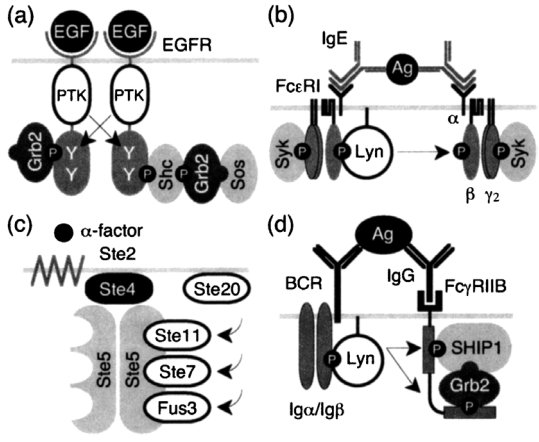

Figure 1. Multicomponent complexes that can form during receptor-mediated signal transduction. (a) Complex formation around EGFR (Jorissen et al., 2003; Schlessinger, 2000). The cytosolic adapters Grb2 and Shc are recruited to the membrane when EGFR tyrosines are autophosphorylated. Grb2 also binds phosphorylated Shc and interacts constitutively with Sos, a guanine nucleutide exchange factor. (b) Complex formation around FcεRI (Kinet, 1999; Turner and Kinet, 1999). Syk, a cytosolic protein tyrosine kinase (PTK), is recruited to the γ chain of FcεRI after phosphorylation of receptor tyrosines by the Src-family PTK Lyn, which is tethered to the membrane and interacts with the β chain of FcεRI via constitutive low-affinity and phosphorylation-dependent high-affinity interactions. (c) Complex formation around Ste5p (Elion, 2001). The kinases Stel1p, Ste7p, and Fus3p constitute a MAPK cascade involved in the mating response of yeast and interact with the scaffold protein Ste5p, which forms homodimers. When α-factor pheromone binds Ste2p, Ste4p, a G protein component, is liberated to interact with Ste5p. Recruitment of Ste5p to the membrane enables membrane-associated kinase Ste20p to phosphorylate Ste11p, which initiates the MAPK cascade. (d) Complex formation around FcγRIIB (March and Ravichandran, 2002). SHIP1, a cytosolic inositol phosphatase, is recruited to the membrane after phosphorylation of FcγRIIB tyrosines. Recruitment of SHIP1 depends on Grb2, which interacts constitutively with SHIP1 and, like SHIP1, interacts with FcγRIIB.

(Fig. 1a and 1b). A brief introductory discussion of the well-studied EGFR and FcεRI systems, both of which have been modeled (in different ways), will provide the necessary background for further discussion and allow us to illustrate the problem of combinatorial complexity, i.e., signal transduction within a vast potential chemical reaction network that arises because signaling molecules can be modified in a number of ways and combine to form complexes in a variety of ways. Much of combinatorial complexity is a direct consequence of conditional multivalent binding.

EGFR-Directed Assembly of Complexes

Growth factors and related molecules trigger cell proliferation and other cellular responses through interaction with cell-surface receptors. A well-studied receptor of this type is EGFR (Jorissen et al., 2003; Schlessinger, 2000; Wiley et al., 2003), the receptor for epidermal growth factor (EGF).

The intracellular nucleus of the complex illustrated in Figure 1a is an EGF-induced dimer of EGFR. This complex can form as a result of processes described roughly as follows. Ligand binding promotes or stablizes interactions

between receptors (Ferguson et al., 2003; Garrett et al., 2002; Ogiso et al., 2002). When two receptors are co-localized via ligand-induced dimer formation, as in Figure 1a, the cytoplasmic kinase domain of one EGFR is able to transphosphorylate various cytoplasmic receptor tyrosine residues of the other EGFR (Jorissen et al., 2003; Schlessinger 2000). Phosphotyrosine-containing sites can be recognized by the cytosolic adapter proteins Grb2 and Shc (Batzer et al., 1994; Okabayashi et al., 1994), which are recruited to phosphorylated receptors. When Shc is bound to a receptor, it can be phosphorylated by EGFR (Pelicci et al., 1992). The phosphorylated form of Shc interacts with Grb2 (Rozakis-Adcock et al., 1992), which interacts constitutively with the guanine nucleotide exchange factor Sos (Egan et al.,1993; Li et al., 1993; Rozakis-Adcock et al., 1993). Formation of EGFR·Shc·Grb2·Sos complexes is an important route through which EGFR recruits Sos to the membrane (Sasaoka et al., 1994). Translocation of Sos from the cytosol to the membrane is required for Sos-catalyzed activation of Ras (Boguski and McCormick, 1993), a membrane-tethered GTPase that regulates a mitogen-activated protein kinase (MAPK) cascade. This MAPK cascade, which is similar to the one illustrated in Figure 1c, ultimately activates transcription factors that control gene expression (Chang and Karin, 2001; Treisman, 1996).

The multicomponent complex illustrated in Figure 1a is just one of the many complexes that can potentially form during signaling, in part because the molecules involved in EGFR-mediated signal transduction each have multiple binding sites, as is generally true (Pawson and Nash, 2003). For example, Grb2 binds EGFR and Shc via its Src homology 2 (SH2) domain (Lowenstein et al., 1992; Rozakis-Adcock et al., 1992), and Grb2 binds Sos via its two SH3 domains (Egan et al., 1993; Li et al., 1993; Rozakis-Adcock et al., 1993). The reaction network is complicated not only by multivalent binding and the consequent possibility of a spectrum of multicomponent complexes but also by the conditional activity of component binding sites. For example, Grb2 binds tyrosine residue Y1068 of EGFR only when this residue is phosphorylated. Thus, we must be able to keep track of whether Y1068 is phosphorylated or not if we wish to follow the interaction of EGFR with Grb2. As in this example, many other signaling molecules contain binding sites that can be either on or off depending on phosphorylation/modification state. A further complication is the transitory nature of complexes (Bunnell et al., 2002; Pacini et al., 2000). For example, just as there are enzymes, such as kinases, that modify proteins to turn binding sites on, there are enzymes, such as phosphatases, that reverse these modifications to turn binding sites off (Hunter, 1995). For this reason and others (e.g., short-lived chemical bonds), there is a constant competition during signal transduction in which multicomponent complexes are building up and breaking down. From these considerations, we can start to appreciate the complexity of signaling networks and the difficulties, experimental and theoretical, that must be overcome to develop predictive models of these networks. For any conceivable case, one must consider a spectrum of ephemeral complexes, composed of multiple components, each of which can occupy numerous modification states.

Modeling Early Events in EGFR Signaling

To what extent do the conditional multivalent interactions of signaling molecules complicate modeling? Kholodenko et al. (1999) formulated a mathematical model for early EGFR-mediated signaling events. They primarily focused on the events that lead to recruitment of Sos to the inner membrane. This model includes six proteins and tracks 25 chemical species. It can be reduced to a model that includes five proteins (EGF, EGFR, Grb2, Shc, and Sos) and tracks 18 chemical species if we omit consideration of phospholipase Cγ (PLCγ), which is not required to recruit Sos to the membrane. An extended version of the model that incorporates the MAPK cascade triggered by Sos-activated Ras tracks 94 chemical species (Schoeberl et al., 2002). In both models, reaction dynamics are characterized by a system of coupled ordinary differential equations (ODEs), with the number of ODEs corresponding to the number of chemical species.

The number of equations in the model of Kholodenko et al. (1999) or Schoeberl et al. (2002) is a consequence of the multivalent protein–protein interactions that dominate the EGFR signaling cascade. The reaction network considered in either model, as is typical of protein interaction networks, is larger and more branched than would be expected for a genetic regulatory or metabolic network involving the same number of proteins. For comparison, consider the metabolic network of the red blood cell. A model of this network (Jamshidi et al., 2001) includes 34 ODEs and, unlike the EGFR models, involves a comparable number of enzymes. Gene regulation involves aggregation phenomena as in signal transduction (Ptashne and Gann, 2002), but genetic regulatory networks tend to involve a relatively small set of transcription factors (Thieffry et al., 1998, Shen-Orr et al., 2002), which is reflected in models (Gilman and Arkin, 2002). Thus, in comparison with models for metabolic and genetic regulatory networks, the models of EGFR signaling can be considered large. Nevertheless, these models may not be large enough, because each model explicitly tracks only a fraction of the microscopic chemical species that are potentially involved in the processes considered.

In the model of Kholodenko et al. (1999), the only monomers of EGFR considered are those lacking cytoplasmic modifications and the only dimers of EGFR considered are those in which both receptors are bound to EGF and only a single receptor is in direct contact with, at most, a single adapter protein, Grb2 or Shc but not both. Unaggregated receptors with modified/bound cytoplasmic domains, dimers of EGFR involving EGF-free receptors (Jorissen et al., 2003), and dimers of EGFR in direct contact with more than a single adapter protein (Jiang and Sorkin, 2002), such as the one illustrated in Figure 1a, are among the types of complexes assumed not to form. If we wish to account for all

chemical species that are possible when the protein interaction domains of EGFR are considered to be independent, then a model without PLCγ, incorporating the same scope of interactions considered by Kholodenko et al. (1999), must track, depending on mechanistic assumptions, hundreds to thousands of chemical species.

For example, we can identify 1232 potential chemical species based on the following assumptions about the possible states of the relevant protein domains. The extracellular domain of a receptor can be either free or bound to EGF. The Grb2 binding site on EGFR can be (1) unphosphorylated, (2) phosphorylated, (3) bound to Grb2, or (4) bound to Grb2 associated with Sos. The Shc binding site on EGFR can be (1) unphosphorylated, (2) phosphorylated, (3) bound to Shc, (4) bound to phosphorylated Shc, (5) bound to Shc associated with Grb2, or (6) bound to Shc associated with Grb2 and Sos in complex. Thus, from combinatorics, there are $2 \times 4 \times 6 = 48$ species containing a single receptor, an equal number of species containing a symmetric dimer of EGFR, and $\binom{48}{2} = 1128$ species containing an asymmetric dimer of EGFR. In addition to these receptor-containing species, there are seven cytosolic chemical species and free extracellular EGF.

Are all of these chemical species important? Probably not. Nevertheless, a consideration of all possible chemical species implied by mechanistic assumptions, at least initially, would seem valuable. One reason is that there is usually no basis for discarding chemical species from consideration. One can make complicated assumptions (e.g., that complexes like the one illustrated in Figure 1a cannot form) and derive a minimalist model, or one can make minimalist assumptions and derive a complicated model and then try to deal with it. An advantage of a complicated model is the possibility of predicting, on the basis of reaction dynamics, which molecular complexes are formed appreciably and which reaction routes are prevalent, as in the theoretical study of Levchenko et al. (2000). Further motivation to consider models that incorporate all complexes implied by known molecular interactions is provided by the advent of proteomic methods for monitoring protein modifications and protein–protein interactions on a multiple protein scale (Aebersold and Mann, 2003; Mann and Jersen, 2003; Meyer and Teruel, 2003). Proteomic studies have revealed that activated receptors, including EGFR, associate with a large number of proteins (Blagoev et al., 2003; Bunnell et al., 2002; Husi et al., 2000), which implies a spectrum of protein complexes. To make sense of such observations using a mathematical model, the complexity of the data must be matched by the complexity of the model used to analyze the data.

The potential need to consider a large number of chemical species simply to model early membrane-proximal signaling events is not at all unique to the EGFR system. In fact, this problem of combinatorial complexity is common if not universal and has been recognized by a number of modelers. For example, Endy and Brent (2001) pointed out that the interactions of Ste5p, Ste11p, Ste7p, and Fus3p, illustrated in Figure 1c, can lead to the formation of 25,666 distinct chemical species, and Arkin (2001) mentioned that the tumor suppressor protein p53 (Vogelstein et al., 2000) can occupy $2^{27} = 134{,}217{,}728$ phosphoforms, because it contains 27 sites at which phosphate can be added or removed. Wofsy et al. (1997) made similar comments concerning the phosphorylation states of aggregated immunoreceptors. Of course, we do not expect estimates about the possible number of phosphoforms of a protein or a protein complex to reflect the number of phosphoforms that are realized during signal transduction, which is impossible, for example, when the number of potential phosphorylation states exceeds the number of molecules available for phosphorylation. Direct experimental observations also indicate that the realizable diversity of molecular phosphoforms is sometimes limited. For example, Kersh et al. (1998) observed that only certain ligands induce complete phosphorylation of the ζ chain of the T cell receptor (TCR). Nevertheless, for each molecule subject to phosphophorylation that one might wish to consider, it seems reasonable to expect multiple phosphoforms. It also seems reasonable to expect identification of the relevant phosphoforms to be clouded by the combinatorial possibilities. Recently, Bray (2003) called attention to the problem of combinatorial complexity, dubbing it molecular prodigality. What are the practical modeling approaches to this problem? To answer this question, we now consider the FcεRI system and a mathematical model that has been developed for early events in FcεRI-mediated signal transduction.

FcεRI-Directed Assembly of Complexes

The intracellular nucleus of the multicomponent complex illustrated in Figure 1b is an antigen-induced dimer of FcεRI, which forms long-lived complexes with IgE antibody and triggers allergic reactions. For an overview of the FcεRI system, see Kinet (1999) and Turner and Kinet (1999). FcεRI is quite different from EGFR. Signaling by FcεRI can be triggered by any multivalent antigen (e.g., a foreign protein) that is recognized by the variable antigen-combining sites of IgE in complex with FcεRI. Consequently, because receptor aggregation depends on ligand properties, a spectrum of receptor aggregates, not just dimers, can form as a result of ligand-receptor interaction. Furthermore, unlike EGFR, FcεRI lacks intrinsic kinase activity, although phosphorylation of FcεRI is just as critical for generating signals as in the case of EGFR. Despite the functional and mechanistic differences between EGFR and FcεRI, signaling by both receptors involves the directed assembly of intracellular complexes.

The intracellular components of the complex illustrated in Figure 1b are the cytoplasmic polypeptide chains of FcεRI and two PTKs, Lyn and Syk. FcεRI when expressed on mast cells and basophils, is a tetrameric complex that consists of an α chain, which contains the extracellular IgE binding site, a β chain, which interacts with the Src-family PTK Lyn, and two identical disulfide-linked γ chains,

which interact with the PTK Syk. The γ chain is essential for signaling, while the β chain is dispensable but acts as a signal amplifier. The β and γ chains each contain a single cytoplasmic ITAM. The β and γ ITAMs, when phosphorylated, serve as binding sites for Lyn and Syk. Lyn, which is anchored to the inner cell membrane, interacts with the β chain in two ways: weakly through its unique domain when the β chain is unphosphorylated and more tightly through its SH2 domain when the β ITAM is phosphorylated. Syk binds the γ ITAM with high affinity through its two SH2 domains when the γ ITAM is doubly phosphorylated.

A critical early event in FcεRI signaling is the activation of Syk. FcεRI triggers activation of Syk roughly as follows (Kinet, 1999; Turner and Kinet, 1999). Upon ligand-induced receptor aggregation, the β and γ ITAMs are phosphorylated by receptor-associated Lyn. Phosphorylation of the γ ITAM recruits cytosolic Syk to receptors. Syk is then phosphorylated by Lyn and by itself at multiple sites, with Syk being primarily responsible for phosphorylating tyrosine residues in its activation loop. Autophosphorylation of Syk is required for full Syk activity and downstream Syk-dependent events (Zhang et al., 2000).

Modeling Early Events in FcεRI Signaling

Like early events in EGFR signaling, early events in FcεRI signaling have been modeled. A mathematical model for FcεRI-mediated activation of Syk has been developed based on the understanding of FcεRI summarized above (Faeder et al., 2003; Goldstein et al., 2002). The model characterizes the interactions of four molecules: a bivalent ligand that recognizes a single receptor site (e.g., a chemically cross-linked dimer of IgE), the receptor, Lyn, and Syk. Processes considered in the model include ligand-induced aggregation of receptors, reversible binding of Lyn and Syk to receptor subunits, the context-dependent kinase activities of Lyn and Syk, and phosphatase activity, with phosphatases being considered implicitly. The model tracks 354 chemical species in a network of 3680 unidirectional reactions.

The 354 chemical species arise as follows. The receptor is considered to consist of three domains: an extracellular α domain that binds ligand, an intracellular β domain that binds Lyn, and an intracellular γ domain that binds Syk. The α, β, and γ domains are allowed to occupy, respectively, two states (free or bound), four states (naked, phosphorylated, or bound to Lyn loosely or tightly), and six states (naked, phosphorylated, or bound to any of four different phosphoforms of Syk). Thus, from combinatorics, there are $2 \times 4 \times 6 = 48$ monomeric receptor species, $4 \times 6 = 24$ symmetric dimeric receptor species (note that the α domains of both receptors in a dimer are necessarily bound), and $\binom{24}{2} = 276$ asymmetric dimeric receptor species. In addition, there are six non-receptor species (free ligand, free Lyn, and the four phosphoforms of Syk in the cytosol).

How was the FcεRI model, consisting of 354 ODEs and including a rate constant for each of 3680 reactions, formulated? To characterize the transitions among the chemical species, a set of reaction rules was specified. The rules represent a description of the local activities of protein domains in the FcεRI system, which are characterized by a relatively small set of rate constants (21) and stereochemical and spatial constraints (Faeder et al., 2003). In other words, a description of protein domain activities and interactions was used to characterize the interactions of a set of whole molecules that each contain multiple protein domains. For each of the 354 chemical species, the domain-based reaction rules were used, with the aid of a computer, to exhaustively enumerate and classify the possible binding and enzymatic reactions. During this process of network generation, each reaction deemed possible was assigned a rate constant specified for its class of domain activity, with reactions of the same class being assigned the same rate constant. For example, a single rate constant was used to characterize each of the 24 distinct reactions that involve association of free ligand with FcεRI. (Note that there are 24 reactions because there are 24 possible states of a receptor that is unbound to ligand). Although the total number of reactions in the model is large for combinatorial reasons, the number of reaction types, which is related to the number of molecular domains, is relatively small. The number of parameters in the model is comparable to the number of molecular domains, not the number of chemical species or reactions.

The software used to accomplish the task of generating the FcεRI reaction network is available at cellsignaling.lanl.gov. The output of this software is a list of reactions that can be used to automatically build either a system of dynamic mass balance equations (i.e., ODEs) or a Monte Carlo stochastic simulation algorithm (SSA) (Gillespie, 1976; 1977). This list of reactions provides a description of the FcεRI model that is complete in microscopic detail for the scope of domain interactions considered. Of course, generation of the reaction network is based on the assumption that the activity of each molecular domain is independent of its context except as explicitly specified, and we are unlikely to know all the context-dependent constraints that are relevant for a large reaction network. However, this rule-based domain-oriented approach to modeling offers a starting point that matches the level of complexity likely to be involved in signaling and from which model refinement can begin.

THE DOMAIN-ORIENTED MICROSCOPIC VIEWPOINT

The FcεRI model (Faeder et al., 2003; Goldstein et al., 2002) represents a domain-oriented microscopic view of signal transduction. We use the term ''domain oriented,'' because the mathematical description accounts for the interactions, modifications, and activities of molecular domains, and we say ''microscopic,'' because the description tracks the full spectrum of molecular complexes implied by the specified domain interactions. The level of modeling detail is less than atomistic but greater than molecular. The FcεRI model differs fundamentally from the usual sort of dynamic models that are currently being developed for signal transduction

systems (cf., Kholodenko et al., 1999; Schoeberl et al., 2002), which provide less complete descriptions of the possible interactions among signaling molecules and the possible multicomponent signaling complexes. Although much can be learned from simple models, we feel many of the interesting questions that one can ask about signal transduction are best addressed using mathematical models that incorporate the domain-oriented microscopic viewpoint.

The level of detail in a domain-oriented model is consistent with the large body of evidence indicating that molecular domains, not molecules, are the fundamental elements of signal transduction systems (Pawson and Nash, 2003). A model that incorporates detail at the level of molecular domains can be used to interpret the results of most types of experiments that are performed to study the behavior of signal transduction systems. The experimentalist typically alters or replaces domains and deletes or overexpresses molecules. These perturbations can be readily mapped to parametric and structural variations of a domain-oriented model, just as experimental readouts can be readily mapped to model variables.

Modeling at the domain level also facilitates stepwise model development and validation. Because the parameters in a domain-oriented model are concentrations and single-site rate constants for domain activities, these parameters are independent of systemic properties and, in principle, can be measured experimentally in isolation. After parameters of component interactions and activities are measured, a systems-level model incorporating these interactions and activities can be built and tested against measurements of system behavior. Also, if a parameter value is chosen so that a model is consistent with observed system behavior, the value of this parameter can then be compared with the value determined in an independent experiment. In our opinion, the main difficulty involved in parameterizing a signaling network is a lack of understanding of how the interactions, modifications, and activities of molecular domains are affected by context, i.e., our ignorance of, for example, induced (conformational) changes that affect activity/affinity (Chigaev et al., 2001; Shimaoka et al., 2003), cooperative interactions (Prehoda and Lim, 2002), effects of spatial compartmentalization (Haugh et al., 1999), steric clashes (Nishimura et al., 1993), etc. Steric limitations could, perhaps in many cases, be dealt with through a combination of homology modeling and molecular docking studies (Smith and Stemberg, 2002; Tovchigrechko et al., 2002), because three-dimensional structures are available for representatives of a number of the protein domains involved in signaling (Bateman et al., 2002; Berman et al., 2000) and, in the absence of an experimentally determined structure, ab initio structure predictions are available for many of the major protein families (Bonneau et al., 2002).

Domain-oriented models of signal transduction present challenges. They lead immediately to the problem of combinatorial complexity, the need to account in some way for a vast chemical reaction network. For some systems, the possible chemical species can be enumerated, local domain-based rules can be defined and used to generate the underlying reaction network for molecular interactions, and standard numerical methods can be applied to analyze the model obtained without major computational difficulties (Faeder et al., 2003; Goldstein et al., 2002). For other systems, such as those in which polymerization reactions are possible, the scale of the computational challenge is larger. It can be quite difficult to formulate a model for an aggregating system that involves intermolecular chain-propagation and intramolecular ring closure reactions (Bray and Lay, 1997; Dembo and Golstein, 1978; Goldstein and Perelson, 1984; Macken and Perelson, 1985; Perelson, 1984; Perelson and DeLisi, 1980; Posner et al., 1995b), and these reactions can definitely arise during signal transduction. The ternary complex of FcγRIIB, SHIP1, and Grb2 (Fong et al., 2000), illustrated in Fig. 1d, provides an example of a complex formed through ring closure. In theory, FcγRIIB, SHIP1, and Grb2 can also form aggregate chains. For example, the complexes FcγRIIB·SHIP1 and FcγRIIB·Grb2 might combine through the interaction of SHIP1 and Grb2 (Osborne et al., 1996). Other systems that are likely to pose computational difficulties include those in which the number of potential chemical species greatly exceeds the number of molecules available to populate the various species. As discussed by Endy and Brent (2001), the MAPK cascade of Fig. 1c is an example of such a system.

A promising modeling approach for the types of systems mentioned above might be simultaneous network generation and Monte Carlo simulation of reaction dynamics along the lines suggested by Faulon and Sault (2001) for chemical systems described by the Dugundji-Ugi model (Ugi et al., 1993). This approach is feasible for signal transduction systems if one can formulate rules for network generation, because it is possible to implement a SSA without full knowledge of the elements in a reaction network. To advance a Monte Carlo simulation of chemical reaction dynamics, we require only knowledge of the chemical states that are populated at the current time, the empty chemical states that neighbor these states, and the reaction propensities that characterize transitions among the states in play (Faulon and Sault, 2001; Gillespie, 1976, 1977). When a chemical state becomes populated for the first time, reaction rules can be used to modify the governing SSA, if necessary, to account for the newly occupied state and any elements of the reaction network that are newly accessible, i.e., the rules can be applied to update the probability distributions used in the SSA to select reaction events. Other approaches that might prove useful include agent-based models (Le Novère and Shimizu, 2001; Morton-Firth and Bray, 1998) and formal mathematical descriptions of molecular interactions (Priami et al., 2001).

EFFECTS OF COMPLEX FORMATION ON SIGNAL TRANSDUCTION

Why is the directed assembly of multicomponent complexes so prevalent in signal transduction systems and what are the

functional consequences of complex formation? In many cases, the assembly of a complex brings an enzyme into proximity of a substrate, which increases the local substrate concentration and, thus, the substrate-specific activity of the enzyme (DeLisi, 1980; Haugh and Lauffenburger, 1997; Kholodenko et al., 2000). Ptashne and Gann (2002) have called this phenomenon regulated recruitment and have identified it as a common mechanism by which specificity is imposed on enzymes, especially in regulatory systems. Consider, for example, the MAPK cascade of Figure 1c. Recently, Park et al. (2003) have shown that the specificity of this cascade is due largely to regulated recruitment, in that the cascade still responds, specifically, to α-factor pheromone when heterologous protein–protein interactions replace the native interactions between Ste5p and its binding partners. Thus, simple recruitment of the MAP kinases to Ste5p is sufficient for signal transduction. Proper positioning of the kinases via native interactions contributes to signaling but is not absolutely required. Of course, stereochemical constraints can be critical. For example, steric constraints are believed to explain why some ligands that induce aggregation of FcεRI, which is usually sufficient for signaling, fail to stimulate cellular responses (Harris et al., 1997; Paar et al., 2002; Posner et al., 1995a).

To say that complex assembly during signal transduction controls enzyme activity is just to scratch the surface. The functional consequences of complex assembly are multifaceted (Burack et al., 2002; Ferrell, 1998; Kholodenko et al., 2000; O'Rourke and Ladbury, 2003; Prehoda and Lim, 2002). Below, we discuss some of the surprising ways that ligand-induced complex formation can influence receptor-mediated signal transduction. We also review results from modeling studies, primarily of FcεRI, which indicate that the assembly of complexes can determine how cellular responses are influenced by ligand-receptor binding kinetics and a ligand stimulus. In addition, we consider how the dynamics of complex assembly can affect the response of a system to a perturbation of the system's structure. The examples we discuss illustrate how the qualitative behavior of a signal transduction system can depend on quantitative factors.

Energy-Driven Complex Assembly Introduces Kinetic Proofreading

One of the more influential models of signal transduction has been the simple kinetic proofreading model of McKeithan (1995), which has prompted both experimental (Liu et al., 2001; Torigoe et al., 1998) and theoretical (Faeder et al., 2003; Hlavacek et al., 2001; 2002) studies of the effects of ligand-receptor binding kinetics on FcεRI signaling. According to the model of McKeithan (1995), a receptor must undergo a series of modifications before generating a productive signal. These modifications are imagined to involve receptor phosphorylation, an ATP-consuming process, and phosphorylation-dependent assembly of signaling complexes. If a ligand dissociates before receptor modifications are completed, the generation of a productive signal is prevented. Thus, one expects that slowly-dissociating ligands will generate stronger cellular responses than rapidly dissociating ligands. Moreover, signaling events that require a smaller number of receptor modification steps should be less sensitive to ligand-binding kinetics than signaling events that require more receptor modification steps (Hlavacek et al., 2002).

In the case of FcεRI, these predictions have been confirmed for certain cellular responses (Torigoe et al., 1998). However, a particular late response to FcεRI signaling, synthesis of a chemokine mRNA, has been found to be insensitive to differences in ligand-receptor binding kinetics (Liu et al., 2001). In light of this result, the model of McKeithan (1995) was extended in several ways to bring this phenomenonological model, developed with TCR signal transduction in mind, into closer correspondence with molecular mechanisms, particularly of the FcεRI system, and these model extensions were studied to determine their potential impact on kinetic proofreading (Hlavacek et al., 2001; 2002). It was found that the involvement of a cytosolic messenger, such as a transcription factor that translocates to the nucleus after receptor-mediated activation in the cytosol, can explain how slowly and rapidly dissociating ligands can stimulate similar cellular responses: in this case, both ligands cause messenger activation to saturate. Signaling by receptors in an intermediate state of modification can also explain how ligands with different kinetic properties might trigger similar responses or even responses in which the expected sensitivity to ligand-receptor binding kinetics is reversed (Hlavacek et al., 2002).

Recently, kinetic proofreading was examined using the domain-oriented FcεRI model discussed earlier (Faeder et al., 2003). The network structure of this model differs dramatically from the linear cascade considered in the model of McKeithan (1995), but kinetic proofreading still emerges (Faeder et al., 2003). This result, because it is derived from a mechanistic description of FcεRI signaling and not a phenomenological model, provides theoretical support for McKeithan's intuitive insight that the energy-driven reactions of signal transduction can cause cellular responses to depend on ligand-receptor binding kinetics.

The model of Faeder et al. (2003) also allows new insights into kinetic proofreading. In Figure 2, we can compare the cellular responses that the model predicts for slowly and rapidly dissociating ligands. The comparison is controlled, as in experimental comparisons (Liu et al., 2001; Torigoe et al., 1998), in that the ligands differ intrinsically only in the dissociation rate constant that characterizes ligand-receptor binding and the concentrations of the two ligands are such that receptor aggregation is the same in each case at equilibrium. As can be seen, after a transient, Syk autophosphorylation is more extensive when signaling is stimulated by the slowly-dissociating ligand, which is consistent with the model of McKeithan (1995). Likewise, phosphorylation of the γ ITAM of FcεRI is more extensive, but only slightly so. In contrast, during the initial transient, the rapidly dissociat-

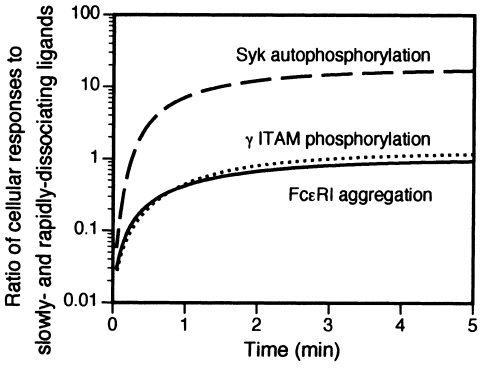

Figure 2. Sensitivity of cellular responses to ligand-receptor binding kinetics. Aggregation of FcεRI (solid curve), autophosphorylation of Syk (broken curve), and Lyn phosphorylation of the γ ITAM of FcεRI (dotted curve) are followed as a function of time after ligand stimulation. Two bivalent ligands that recognize a single receptor site and that have different kinetic properties are considered. The first ligand forms short-lived bonds with receptors (the dissociation rate constant is 0.5 s^{-1}) and is introduced at total concentration 7.0×10^{-9} M. The second ligand forms long-lived bonds with receptors (the dissociation rate constant is 0.05 s^{-1}) and is introduced at total concentration 7.6×10^{-11} M. Both ligands are characterized by a forward rate constant for ligand-receptor binding of 10^6 M^{-1} s^{-1} and a forward rate constant for receptor crosslinking, scaled by the total receptor surface density, of 0.5 s^{-1}. Calculations are based on the model and parameter values, except as noted above, of Faeder et al. (2003). Each curve indicates the response stimulated by the slowly dissociating ligand relative to that stimulated by the rapidly dissociating ligand. Note that the two ligand stimuli each induce receptor aggregation to the same extent at steady state, which is approached within 5 min.

ing ligand is more effective than the slowly dissociating ligand at eliciting phosphorylation of the receptor and autophosphorylation of Syk. This result is obtained because receptor aggregation, as indicated in Figure 2, approaches equilibrium faster in the case of the rapidly dissociating ligand than in the case of the slowly dissociating ligand (Hlavacek et al., 2002). Thus, receptor aggregates, which facilitate receptor and Syk phosphorylation, are more abundant initially in the case of the rapidly dissociating ligand, and this fact compensates for the lower stimulatory capacity of this ligand that is manifested at later times. As recent studies and Figure 2 indicate, ligand recognition based on kinetic proofreading is more complicated than originally thought, depending not simply on the lifetime of a ligand-receptor bond but also on the mechanistic details of signal transduction, quantitative factors, and perhaps even the kinetics of kinetic proofreading.

Complex Assembly Can Determine If a Ligand Stimulus Induces or Represses a Cellular Response

Burack and Shaw (2000) pointed out that scaffolds, like Ste5p (Fig. 1c), when overexpressed could have either negative or positive effects on signal transduction as a result of multivalent binding, as in immune precipitation (Day, 1990). Contemporaneously, Levchenko et al. (2000) developed a mathematical model for a MAPK cascade that explicitly incorporates a scaffold molecule and showed that there is an optimal scaffold concentration for signal transduction, which can be attributed to multivalent binding. At low scaffold concentration, a scaffold nucleates complexes, which enhances signal transduction. The scaffold brings its two binding partners, enzyme and substrate, together. Up to a point, an increase in scaffold concentration causes an increase in the number of scaffold-associated complexes that contain both enzyme and substrate. However, after this point, an increase in scaffold concentration is inhibitory, because it becomes unlikely that both binding partners of the scaffold will be bound to the same scaffold molecule. The scaffold, when present in excess, acts to separate its binding partners. In a related earlier work, Bray and Lay (1997) discussed the potential inhibitory effect of a multivalent protein on formation of multimeric complexes.

Similar potential for complex assembly to negatively or positively affect signaling, as a result of multivalent binding, has also been predicted on the basis of the model for FcεRI-mediated activation of Syk (Goldstein et al., 2002). In Figure 3, autophosphorylation of Syk at steady state is shown as a function of bivalent ligand concentration for two hypothetical cells with different concentrations of Syk. The first cell, in which the number of Syk molecules matches the number of receptors, corresponds to the bell-shaped curve. The second cell is identical to the first cell with the exception that Syk is l0-fold less abundant. As illustrated, varying the concentration of a component in a signal transduction system can have nonlinear effects on signaling that are even more

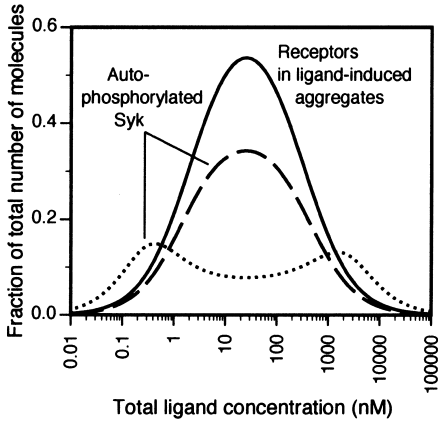

Figure 3. Diverse nonlinear ligand dose-response curves. Steady-state levels of FcεRI aggregation (solid curve) and Syk autophosphorylation (broken and dotted curves) are shown as a function of total ligand concentration. The ligand considered is the slowly dissociating ligand of Figure 2. The broken and dotted curves correspond to cases in which there are 4×10^5 and 4×10^4 molecules of Syk per cell. In each case, for purposes of illustration, we have increased the total amount of available Lyn from the empirical 2.8×10^4 molecules per cell (Wofsy et al., 1997) to 4×10^5 molecules per cell, which enhances ligand-induced receptor phosphorylation and makes the shape of the ligand dose-response curve for Syk autophosphorylation more sensitive to the abundance of Syk. Calculations are based on the model and parameter values, except as noted avove, of Faeder et al. (2003).

complicated than those discussed in the paragraph above. The ligand dose-response curve for Syk autophosphorylation can exhibit not only a maximum but also two maxima depending on the abundance of Syk.

Complex input–output behavior arises because, in the model, the mechanism of Syk autophosphorylation is trans-phosphorylation (Faeder et al., 2003). Thus, Syk autophosphorylation depends on juxtaposition of two Syk molecules, which depends on the number of receptor aggregates with two Syk binding sites (i.e., aggregates in which two receptors contain a phosphorylated γ ITAM), which, in turn, depends on the concentration of bivalent ligand. In the case of the first hypothetical cell, Syk autophosphorylation simply follows ligand-induced receptor aggregation, which peaks at an optimal ligand concentration. In contrast, in the case of the second hypothetical cell, because of the lower concentration of Syk, receptor dimers able to mediate juxtaposition of Syk become available in excess before receptor aggregation peaks. When these dimers are in excess, it becomes unlikely that two molecules of Syk will join the same receptor aggregate. As a result, juxtaposition (and autophosphorylation) of Syk peaks before receptor aggregation is maximal, and Syk autophosphorylation fades as the extent of receptor aggregation increases. Subsequently, Syk autophosphorylation recovers, peaking a second time, as receptor aggregation decreases as a result of excess ligand. It seems unlikely that the type of system behavior illustrated in Figure 3 could be predicted without quantitative characterization of the factors that govern the revelant protein–protein interactions and without a mathematical model for these interactions.

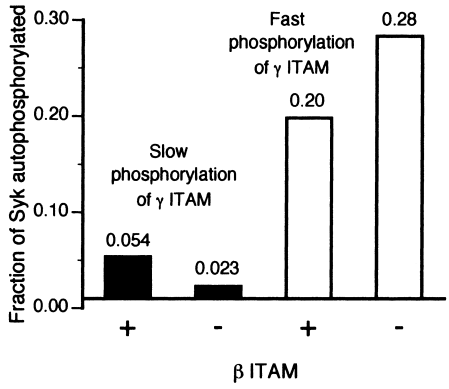

Figure 4. Changing the rate at which Lyn phosphorylates the γ ITAM of FcεRI can switch the behavior of the β ITAM of FcεRI from an amplifier to an attenuator of Syk autophosphorylation. This plot shows the predicted effect of eliminating recruitment of Lyn to receptors via Lyn interaction with the phosphorylated β ITAM for two cases. The first case, in which Lyn phosphorylation of the γ ITAM is slow, is consistent with the experimental observation that the β ITAM acts as an amplifier of FcεRI signaling (Lin et al., 1996) and is based on the model and parameter values of Faeder et al. (2003). In the second case, Lyn phosphorylation of the γ ITAM is fast; the rate at which Lyn phosphorylates the γ ITAM has been increased to match the rate at which Lyn phosphorylates the β ITAM (cf., Faeder et al., 2003). The vertical axis indicates the level of Syk autophosphorylation after 30 min of stimulation with 1.6 n*M* of chemically crosslinked IgE dimers.

The Dynamics of Intracellular Complexes Can Influence Signaling

Lin et al. (1996) observed that the β ITAM of FcεRI acts as an amplifier of signaling, in that signaling is attenuated when the β ITAM is absent. Surprisingly, analysis of the FcεRI model has revealed that the β ITAM can act as either an amplifier or attenuator of signaling (Faeder et al., 2003), which is explained as follows. The amplifier function of the β ITAM depends on a balance between competing processes that have been called kinetic proofreading and serial triggering in the context of TCR signaling (Lanzavecchia et al., 1999). The bond between the SH2 domain of Lyn and a phosphorylated β ITAM is relatively long-lived compared to the weak bond formed between the unique domain of Lyn and the β chain. By allowing more time for Lyn to trans-phosphorylate neighboring receptors, the long-lived SH2-ITAM bond can promote overall receptor phosphorylation if Lyn is slow-acting (i.e., unable to catalyze significant phosphotransfer in the time allowed by the weak bond between Lyn and β). This outcome is the kinetic proofreading effect. However, the SH2-ITAM interaction also limits the number of receptors bound by Lyn within a given period of time. This ITAM-dependent sequestration of Lyn can limit overall receptor phosphorylation if Lyn is fast-acting, especially because the amount of Lyn available to interact with receptors is limited (Torigoe et al., 1997; Wofsy et al., 1997). This outcome is the serial triggering effect. As illustrated in Figure 4, the balance between kinetic proofreading and serial engagement can be shifted by varying the rate constant for Lyn phosphorylation of the γ ITAM.

CONCLUSIONS AND FUTURE DIRECTIONS

Signal transduction systems consist of interacting multivalent molecules, the domains of which have conditional activities. Understanding conditional multivalent binding reactions, and the assembly of multicomponent complexes directed by these reactions, is central to our understanding of signal transduction. To achieve this understanding, we need mathematical models that account for the interactions, modifications, and activities of molecular domains, including the potential array of chemical species that can form during signal transduction. If we are to engineer cells for biotechnological applications in complex environments or develop new strategies to intervene in signaling for therapeutic purposes, we must begin to embrace the challenges of modeling signal transduction from the domain-oriented microscopic viewpoint. The technology is available or will be available soon not only to perturb signal transduction systems systematically but also to monitor signal transduction events comprehensively. For the most part, models have yet to be developed that approach the needs of this coming challenge. To develop and exploit these models, we expect that new computational tools and methods of

analysis will be needed. In addition, the efforts of modelers and quantitative experimentalistists will have to be tightly integrated.

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