Models, Simulations, and Parameters

The demonstration that a single Lyn molecule can initiate phosphorylation of tyrosines on receptors in an aggregate depends on the comparison of dose-response data with predictions of two competing mathematical models. In model A, phosphorylation of aggregated (dimerized) receptors is initiated when the aggregate contains one Lyn molecule, associated with one of the paired receptors. That single Lyn transphosphorylates the other receptor. In model B, both receptors in a dimer must be associated with Lyn for transphosphorylation to proceed. Here we demonstrate the structure of the models by presenting model A in detail. Model A has 14 possible states for the receptor, illustrated in Fig. 4. Their concentrations, along with the concentrations of free Lyn and free ligand, are determined by three conservation laws and 13 coupled, nonlinear ordinary differential equations, given below. The legend to Fig. 4 defines the notation we use for these concentrations, and for the rate constants that govern transitions among the states. In addition, R_T, L_T, and C_T denote the total concentrations of receptor (FcεRI), Lyn, and ligand (IgE dimers), respectively.

The conservation equations are

$$R_T = \sum R_{ij} + 2\sum P_{ijk}$$

$$L_T = L + R_{01} + R_{11} + P_{010} + P_{110} + P_{101} + P_{201} + 2(P_{020} + P_{111} + P_{202})$$

$$C_T = C + R_{10} + R_{11} + \sum P_{ijk}.$$

The system of coupled ordinary differential equations for concentrations of the distinct receptor complexes is

$$dR_{01}/dt = [k_{+L}LR_{00} - k_{-L}R_{01}] - [2k_{+1}CR_{01} - k_{-1}R_{11}] - [k_{+2}R_{01}R_{10} - k_{-2}P_{010}] - [k_{+2}R_{01}R_{11} - 2k_{-2}P_{020}]$$

$$dR_{10}/dt = [2k_{+1}CR_{00} - k_{-1}R_{10}] - [k_{+L}LR_{10} - k_{-L}R_{11}] - [k_{+2}R_{00}R_{10} - 2k_{-2}P_{000}] - [k_{+2}R_{01}R_{10} - k_{-2}P_{010}]$$

$$dR_{11}/dt = [2k_{+1}CR_{01} - k_{-1}R_{11}] + [k_{+L}LR_{10} - k_{-L}R_{11}] - [k_{+2}R_{00}R_{11} - k_{-2}P_{010}] - [k_{+2}R_{01}R_{11} - 2k_{-2}P_{020}]$$

$$dP_{000}/dt = [k_{+2}R_{00}R_{10} - 2k_{-2}P_{000}] - [2k_{+L}LP_{000} - k_{-L}P_{010}] + p_{-3}P_{100}$$

$$dP_{010}/dt = [k_{+2}R_{00}R_{11} - k_{-2}P_{010}] + [k_{+2}R_{01}R_{10} - k_{-2}P_{010}] + [2k_{+L}LP_{000} - k_{-L}P_{010}] - [k_{+L}LP_{010} - 2k_{-L}P_{020}] - [p_{+1}P_{010} - p_{-1}P_{110}]$$

$$dP_{020}/dt = [k_{+L}LP_{010} - 2k_{-L}P_{020}] + [k_{+2}R_{01}R_{11} - 2k_{-2}P_{020}]$$

$$dP_{100}/dt = p_{-3}[2P_{200} - P_{100}] - [k_{+L}LP_{100} - k_{-L}P_{110}] - [k^s_{+L}LP_{100} - k^s_{-L}P_{101}]$$

$$dP_{110}/dt = [p_{+1}P_{010} - p_{-1}P_{110}] + [k_{+L}LP_{100} - k_{-L}P_{110}] - [k_{+t}P_{110} - k_{-t}P_{101}] - [k^s_{+L}LP_{110} - k^s_{-L}P_{111}]$$

$$dP_{101}/dt = [k^s_{+L}LP_{100} - k^s_{-L}P_{101}] + [k_{+t}P_{110} - k_{-t}P_{101}] - [k_{+L}LP_{101} - k_{-L}P_{111}] - [p_{+2}P_{101} - p_{-2}P_{201}]$$

$$dP_{111}/dt = [k^s_{+L}LP_{110} - k^s_{-L}P_{111}] + [k_{+L}LP_{101} - k_{-L}P_{111}]$$

$$dP_{200}/dt = -2p_{-3}P_{200} - [2k^s_{+L}LP_{200} - k^s_{-L}P_{201}]$$

$$dP_{201}/dt = [p_{+2}P_{101} - p_{-2}P_{201}] + [2k^s_{+L}LP_{200} - k^s_{-L}P_{201}] - [k^s_{+L}LP_{201} - 2k^s_{-L}P_{202}]$$

$$dP_{202}/dt = [k^s_{+L}LP_{201} - 2k^s_{-L}P_{202}].$$

Initially (before ligand is added), all concentrations are 0 except $R_{00}(0)$ and $R_{01}(0)$. These initial values are taken to be the steady-state values when $C_T = 0$ and are therefore determined by the equations

$$R_T = R_{00}(0) + R_{01}(0)$$

$$L_T = L + R_{01}(0)$$

$$R_{01}(0) = k_{+L}LR_{010}(0)/k_{-L}.$$

To compare the predictions of the model with experiment, we solve the set of ordinary differential equations numerically for all the concentrations and then calculate the fraction of receptors that are phosphorylated, F_p, as a function of time, where

$$F_p = (P_{100} + P_{110} + P_{101} + P_{111} + 2(P_{200} + P_{201} + P_{202}))/R_T.$$

For the simulations we present in *Results* in the main paper, we used the following parameters, estimated as described in ref. 1 from our own experiments, measurements in the literature, and constraints imposed by detailed balance and other relationships among parameters: $k_{+1} = 8 \times 10^4\ M^{-1}s^{-1}$, $k_{-1} = k_{-2} = 1 \times 10^{-5}\ s^{-1}$, $k_{+2}R_T = 100\ s^{-1}$, $k_{+L}L_T = k^s_{+L}L_T = 14\ min^{-1}$, $k_{-L} = 193\ min^{-1}$, $k^s_{-L} = 1\ min^{-1}$, $k_{+t} = 386\ min^{-1}$, $k_{-t} = 2\ min^{-1}$, $p_{+1} = p_{+2} = 50\ min^{-1}$, $p_{-1} = p_{-2} = p_{-3} = 5\ min^{-1}$.

The total concentrations of IgE dimer and FcεRI are those given in *Materials and Methods* in the main paper, converted to nM units.

Model B has two additional parameters, the rates of activation (k_{+a}) and inactivation (k_{-a}) of dimerized Lyn (Fig. 1*B* in main paper). When we keep track of the activation state of each Lyn associated with aggregated receptors, there are 25 possible states for receptors. The model consists of 3 conservation equations and 22 coupled ordinary differential equations. For the simulations, we used $k_{+a} = k_{-a} = 1\ min^{-1}$.

Predictions of both models do not depend sensitively on the rate constants governing processes that occur on a faster time scale than that of the ligand binding from solution. In particular, the value we took for the forward rate of receptor crosslinking, $k_{+2}R_T = 100\ s^{-1}$, is the calculated diffusion limit, but the predicted levels of phosphorylation are essentially unchanged if the value is up to three orders of magnitude lower. The rates of association and dissociation of Lyn with both the phosphorylated and the nonphosphorylated receptor, rates of phosphorylation and dephosphorylation, and rates of activation and inactivation of Lyn in model B, are also in the category of parameters that can change by at least an order of magnitude without changing quantitative predictions significantly.

1. Wofsy, C., Torigoe, C., Kent, U., Metzger, H. & Goldstein, B. (1997) *J. Immunol.* **94,** 77–80.

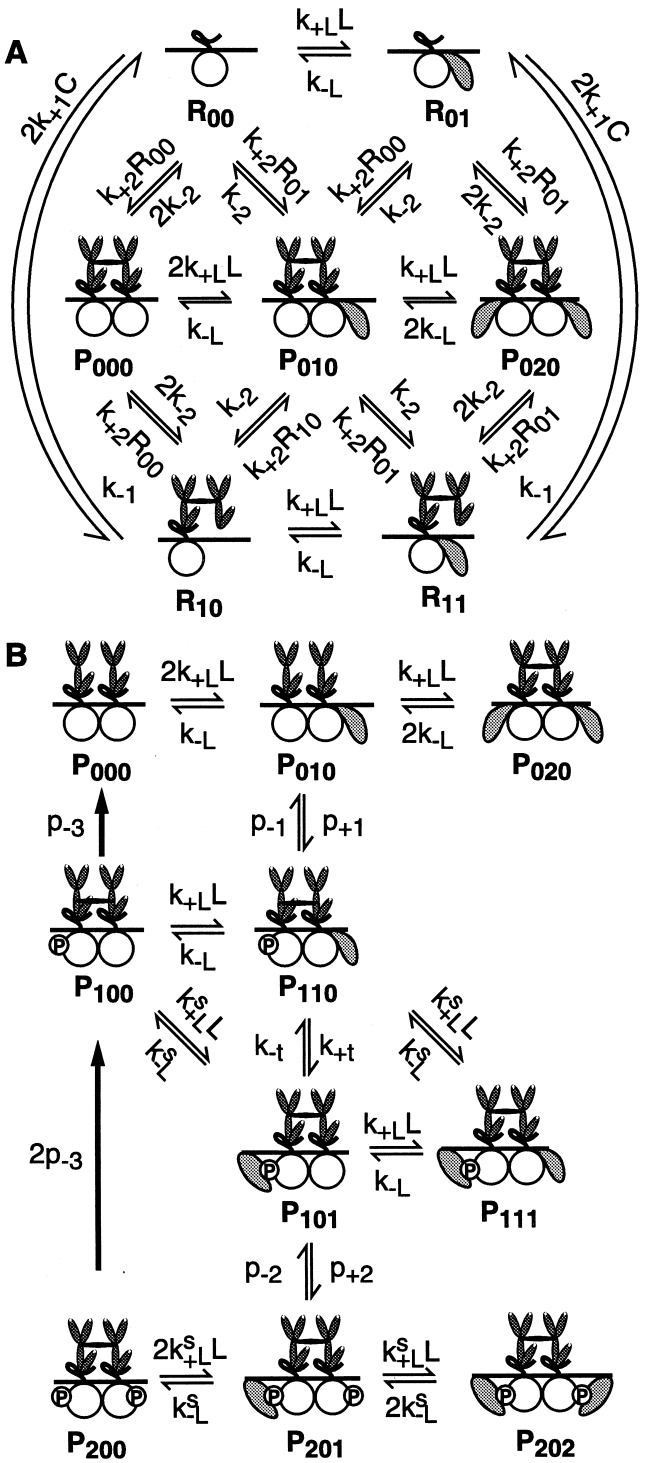

FIG. 4. States of the receptor, and transition rates, for model A, in which a single Lyn molecule can transphosphorylate receptors in an aggregate. *A* includes all of the reactions leading to the formation of receptor aggregates. Aggregates consist of two FcεRI, bound to the two IgE molecules in a covalently linked dimer. *B* includes all of the reactions of aggregated (dimerized) receptors, under conditions where the break-up of aggregates is negligible. This is the case for the experiments described in the main paper, where cells are exposed to dimeric IgE for up to 30 min. The mean time IgE stays bound to FcεRI is on the order of a day. We use the following notation for concentrations of the states and for rates of transition between states. R_{ij} denotes the concentration of unaggregated receptors with i dimers of IgE singly bound (i = 0, 1) and j Lyn molecules constitutively associated (j = 0,1). For the dimeric states, P_{ijk} denotes the concentration of receptor dimers containing i phosphorylated receptors (i = 0,1,2), j Lyn molecules bound to nonphosphorylated receptors (j = 0, . . . , 2 − i), and k Lyn molecules bound to phosphorylated receptors (k = 0, . . . , i). C is the concentration of free ligand (IgE dimer) and L is the concentration of free Lyn. The differential equations for the concentrations involve the following single site forward and reverse rate constants: (1) k_{+1} and k_{-1}, for IgE dimers in solution binding to FcεRI on the cell surface; (2) k_{+2} and k_{-2}, for the binding of the second IgE in a dimer to a second receptor; (3) k_{+L} and k_{-L}, for the constitutive association of Lyn with the nonphosphorylated receptor; (4) k^s_{+L} and k^s_{-L}, for the binding of Lyn to a phosphorylated receptor; and (5) k_{+t} and k_{-t}, for the transfer of Lyn between nonphosphorylated and phosphorylated receptors crosslinked by an IgE dimer. The rates of phosphorylation and dephosphorylation are p_{+1} and p_{-1}, when phosphorylation is mediated by a constitutively bound Lyn, and p_{+2} and p_{-2}, when the kinase is bound to a phosphorylated receptor. A phosphorylated receptor with no Lyn bound is dephosphorylated at a rate p_{-3}.