# IgE Receptor Signaling Utilizes Specialized Membrane Domains

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*Abstract*

Our recent studies have led to the hypothesis that signal transduction immediately following aggregation of the high affinity receptor for immunoglobulin E (Fc$\epsilon$RI) on mast cells involves the association of this receptor with specialized domains of the plasma membrane. We have evidence that these membrane domains, which are characterized by their resistance to solubilization by non-ionic detergents, mediate phosphorylation of aggregated receptors by supplying a locally high concentration of active tyrosine kinase, Lyn. Membrane domain-mediated Fc$\epsilon$RI activation points to a model for signal initiation that emphasizes selective protein-lipid interactions to facilitate functional coupling between proteins. This co-compartmentalization that depends on Fc$\epsilon$RI aggregation is a mechanism for signal regulation that is increasingly appreciated as relevant to signaling by other receptors.

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## Detergent-Resistant Membrane Domains

The plasma membrane comprises a large diversity of components which includes glycerophospholipids, sphingolipids, cholesterol, transmembrane proteins, and lipid anchored proteins. There is increasing evidence that these molecules are not uniformly distributed within the bilayer but are clustered to some extent by their physical properties. Protein-protein interactions provide the basis for some specialized membrane domains, e.g., focal adhesion contacts, clathrin-coated pits, or tight junctions. Recent studies demonstrate that lipid-based interactions can also organize distinct regions of the plasma membrane. Brown and Rose (1992) originally characterized a type of membrane domain that could be isolated because of its insolubility in non-ionic detergents such as Triton X-100. These detergent-resistant plasma membrane domains include flask-like structures identified as caveolae with electron microscopy. Caveolae are organized by both lipid- and protein-based inter-

actions that are facilitated by the oligomerization of the structural protein caveolin (Rothberg et al., 1992; Lisanti et al., 1993).

Cells that do not express caveolin, including mast cells and other hematopoietic cells, do not display caveolae on their surface. However, these cells do contain detergent-resistant membrane domains enriched in some of the same molecules as caveolae (Fra et al., 1994). Formation of these membrane domains is probably facilitated by favorable packing of the predominantly saturated acyl chains of certain lipids. Domains isolated from lysed cells are enriched in sphingomyelin, gangliosides, other glycolipids, and cholesterol. As shown in model membrane studies, membrane domains form in the absence of any structural proteins and exist prior to the addition of non-ionic detergents (Schroeder et al., 1994; Ahmed et al., 1997). As isolated from lysed cells by sucrose gradient ultracentrifugation, these domains are enriched in proteins linked to the plasma membrane by glycosyl-phosphatidylinositol (GPI) or by saturated fatty acids. In particular, certain Src-family tyrosine kinases, including Lyn, associate with these membrane domains, dependent on their tandem myristoyl and palmitoyl acyl chains (Shenoy-Scaria et al., 1993).

## Current Working Model For FcεRI Activation in Membrane Domains

Phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic tails of FcεRI subunits is a tightly controlled step in receptor activation. The unaggregated receptors are not phosphorylated, and they associate weakly with certain components of the membrane domains. In the resting state, the domains are probably dynamic in composition and small in size. Aggregation of FcεRI rapidly enhances receptor-domain associations, possibly because the increased valency of FcεRI aggregates leads to cooperative binding to small clusters of domain components. This causes the formation of larger, more stable membrane domains around the receptor clusters. The locally high concentration of Lyn within the domains favors FcεRI phosphorylation. Once phosphorylated, the ITAM of the $\beta$ subunit of FcεRI binds directly to the SH2 domain of Lyn, and this interaction amplifies the signal, possibly by increasing the effectiveness of Lyn (Lin et al., 1996). Lyn-mediated phosphorylation of the ITAM of the $\gamma$ subunit leads to recruitment, phosphorylation, and activation of the tyrosine kinase Syk, which, in turn, initiates activation of downstream signaling pathways (Scharenberg and Kinet, 1995; Beaven and Baumgartner, 1996).

The formation of membrane domains around aggregated FcεRI may facilitate additional signaling steps, including $Ca^{2+}$ mobilization. Other proteins involved in these processes, such as tyrosine phosphatases and serine/threonine kinases and phosphatases may be enriched or excluded from the domains. Enzymes associated with the domains may have restricted access to other membrane-associated substrates. For example, lipid metabolizing enzymes that are recruited to these receptor complexes would have altered substrate access due to the local enrichment of domain-associated lipids (Pike and Casey, 1996; Hinderliter et al., 1997). In addition, these membrane domains may interact directly with the microfilament cytoskeleton which can further regulate FcεRI signaling (Pierini et al, 1996; 1997).

# Experimental Evidence for FcεRI Activition in Membrane Domains

The most compelling evidence for the model of FcεRI receptor activation in membrane domains is the observation that stimulated tyrosine phosphorylation of FcεRI on cells occurs only with receptors that co-isolate with detergent-resistant membrane domains (Field et al., 1997). A large fraction of FcεRI aggregated with an optimal dose of multivalent ligand associate with the isolated domains, whereas unaggregated FcεRI do not. Furthermore, aggregation-dependent association of FcεRI with membrane domains occurs more rapidly than receptor phosphorylation, and this association occurs similarly when stimulated tyrosine phosphorylation is prevented. We have reconstituted the tyrosine phosphorylation of FcεRI in vitro with isolated membrane domains. The tyrosine phosphorylation of the $\beta$ and $\gamma$ receptor subunits does not occur when micellar Triton X-100 is included in the kinase assay. We found that micellar Triton extracts FcεRI from the domains, thereby decoupling the receptor from active Lyn which remains associated with the domains.

Membrane domains that form on intact cells can be fluorescently labeled with lipid analogs or with antibodies to GPI-linked proteins or gangliosides. DiI-$C_{16}$, a fluorescent lipid analog with saturated acyl chains, co-redistributes at the cell surface with aggregated FcεRI and together with the AA4 ganglioside derivatives and the GPI-linked protein Thy-1. The observation that the DiI-$C_{16}$ located in large patches around aggregated receptors is less laterally mobile than that located elsewhere on the plasma membrane (Thomas et al., 1994) suggests that receptor aggregation can cause the coalescence of less fluid membrane domains, a property predicted for detergent resistant membrane domains from model studies (Schroeder et al., 1994; Ahmed et al., 1997).

Further support for the existence of membrane domains on the plasma membrane of intact cells comes from experiments in which the composition of the plasma membrane is altered by sphingomyelin liposome treatment. Confocal fluorescence microscopy reveals that this treatment promotes the formation of large membrane domains even in the absence of receptor aggregation (Holowka et al., 1996), and other experiments show that functional responses to FcεRI aggregation are enhanced under these conditions (Chang et al., 1995). Experiments with cytochalasin D (Pierini et al., 1997) and with liposome treatments (Holowka et al., 1996) indicate that the membrane domains couple to the cytoskeleton, and interference alters receptor-mediated signaling. Ongoing experiments are directed toward elucidating the molecular details underlying these results.

A central question for understanding the mechanism for coalescence of domains with aggregated FcεRI and the possible role of membrane domains in the function of this and other receptors is the structural basis for this association. A significant clue is the detergent sensitivity of the interaction. As first defined for the association of the $\beta$ and $\gamma$ subunits with the subunit of FcεRI by Metzger and colleagues (Kinet et al., 1985), the ratio of detergent to cell lipid is critical for maintaining interactions between aggregated FcεRI and membrane domains following cell lysis (Field et al., 1998). The working model is FcεRI associating weakly with gangliosides or similar domain components. This association could involve either the transmembrane or extracellular portions of the receptor but does not depend on the $\beta$ and $\gamma$ ITAMs. Our view that aggregation of FcεRI co-clusters small patches of domain components and nucleates the formation of stable, active membrane domains is consistent with current biophysical and functional data (Holowka and Baird, 1996).

## Other Receptors

Remaining to be discovered is how the observations summarized here will extend to other cell surface receptors. This issue is particularly relevant for other receptors that are known to utilize Src-family tyrosine kinases in an early signaling step, such as other members of the multichain immune response receptor family. Recent reports that receptor tyrosine kinases, such as the EGF and PDGF receptors, utilize caveolae to generate local activation signals point to the possibility that compartmentalized receptor activation is a more general phenomena for mitogenic receptors (Mineo et al., 1996; Liu et al., 1996). As appreciation of the complexity of cellular signaling networks grows, localization of signaling components becomes increasingly attractive as a mechanism for the cell to interpret and respond rapidly to the many stimuli it receives.

## References


Ahmed, S.N., D.A. Brown, and E. London. 1997. On the origin of sphingolipid/cholesterol rich detergent-insoluble cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. *Biochemistry* 36:10944-10953.

Beaven, M.A., and R.A. Baumgartner. 1996. Downstream signals initiated in mast cells by FceRI and other receptors. *Curr. Opinion Immunol.* 8:766-772.

Brown, D.A., and J.K. Rose. 1992. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* 68:533-544.

Chang, E.Y., Y. Zheng, D. Holowka, and B. Baird. 1995. Alteration of lipid composition modulates Fc-epsilon-RI signaling in RBL-2H3 cells. *Biochemistry* 34:4376-4384.

Field, K.A., D. Holowka, and B. Baird. 1997. Compartmentalized activation of the high affinity immunoglobulin E receptor within membrane domains. *J. Biol. Chem.* 272:4276-4280.

Field, K.A., D. Holowka, and B. Baird. 1998. Structural basis for the association of FcεRI with detergent-resistant membrane domains. Submitted for publication.

Fra, A.M., E. Williamson, K. Simons, and R.G. Parton. 1994. Detergent-insoluble glycolipid microdomains in lymphocytes in the absence of caveolae. *J. Biol. Chem.* 269:30745-30748.

Hinderliter, A.K., A.R.G. Dibble, R.L. Biltonen, and J.J. Sando. 1997. Activation of protein kinase C by co-existing diacylglycerol-enriched and diacylglycerol-poor lipid domains. *Biochemistry* 36: 6141-48.

Holowka, D., and B. Baird. 1996. Antigen-mediated IgE receptor aggregation and signaling: a window on cell surface structure and dynamics. *Annu. Rev. Biophys. Biomol. Struct.* 25:79-112.

Holowka, D, C. Hine, and B. Baird. 1996. Alterations in cellular lipid composition affect the interactions of aggregated FceRI with $p53/56^{lyn}$ and the microfilament cytoskeleton. *FASEB J.* 10:A1214.

Kinet, J.-P., G. Alcaraz, A. Leonard, and H. Metzger. 1985. Dissociation of the receptor for immunoglobulin E in mild detergents. *Biochemistry* 24:4117-4124.

Lin, S., C. Cicala, A.M. Scharenberg, and J.-P. Kinet. 1996. The FcεRIβ subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. *Cell* 85:985-995.

Lisanti, M.P., Z.L. Tang, and M. Sargiacomo. 1993. Caveolin forms a hetero-oligomeric protecomplex that interacts with an apical GPI-linked protein: implications for the biogenesis of caveolae. *J. Cell. Biol.* 123:595-604.

Liu, P., Y. Ying, Y.G. Ko, and R.G.W. Anderson. 1996. Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae. *J. Biol. Chem.* 271:10299-10303.

Mineo, C., G.L. James, E. . Smart, and R.G.W. Anderson. 1996. Localization of epidermal growth factor-stimulated ras/raf-1 interaction to caveolae membrane. *J. Biol. Chem.* 271:11930-11935.

Pierini, L., D. Holowka, and B. Baird. 1996. FcεRI-mediated association of 6-μm beads with RBL-2H3 mast cells results in exclusion of signaling proteins from the forming phagosome and abrogation of normal downstream signaling. *J. Cell. Biol.* 134:1427-1439.

Pierini, L., N.T. Harris, D. Holowka, and B. Baird. 1997. Evidence supporting a role for microfilaments in regulating the coupling between poorly-dissociable IgE-FcεRI aggregates and downstream signaling pathways. *Biochemistry* 36:7447-7456.

Pike, L.J., and L. Casey. 1996. Localization and turnover of phosphatidylinositol 4,5 bisphosphate in caveolin-enriched membrane domains. *J. Biol. Chem.* 271: 26453-56.

Rothberg, K.G., J.E. Heuser, W.C. Donzell, Y.-S. Ying, J.R. Glenney, and R.G.W. Anderson. 1992. Caveolin, a protein component of caveolae membrane coats. *Cell* 68:673-682.

Scharenberg, A.M., and J.-P. Kinet. 1995. Early events in mast cell signal transduction. *Chem. Immunol.* 61:72-87.

Schroeder, R., E. London, D. Brown. 1994. Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. *Proc. Natl. Acad. Sci. USA* 91:12130-12134.

Shenoy-Scaria, A.M., L.K. Gauen, J. Kwong, A.S. Shaw, and D.M. Lublin. 1993. Palmitylation of an amino-terminal cysteine motif of protein tyrosine kinases $p56^{lck}$ and $p59^{fyn}$ mediates interaction with glycosyl-phosphatidylinositol-anchored proteins. *Mol. Cell. Biol.* 13:6385-6392.

Thomas, J., D. Holowka, B. Baird, and W.W. Webb. 1994. Large-scale co-aggregation of fluorescent lipid probes with cell surface proteins. *J. Cell Biol.* 125:795-802.