Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Immunology Letters 92 (2004) 43–49
Immunology Letters
www.elsevier.com/locate/

Review

Transmembrane adaptor proteins in membrane microdomains: important regulators of immunoreceptor signaling

Václav Hořejší*

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Vídenská 1083, 142 20 Prague 4, Czech Republic

Received 14 September 2003; accepted 10 October 2003

Abstract

Membrane microdomains enriched in glycosphingolipids, cholesterol, glycosylphosphatidylinositol-anchored proteins and Src-family kinases (lipid rafts, GEMs) appear to play many important roles, especially in immunoreceptor signaling. Most transmembrane proteins are excluded from these specialized areas of membranes, notable exceptions being several palmitoylated proteins such as the T cell coreceptors CD4 and CD8, and several recently described transmembrane adaptor proteins, LAT, non-T cell activation linker (NTAL)/linker for activation of B cells (LAB), phosphoprotein associated with GEMs (PAG)/Csk-binding protein (Cbp) and LIME. All these molecules possess a very short N-terminal extracellular peptide (4–17 amino acids), transmembrane segment followed by a palmitoylation motif (CxxC) and cytoplasmic domain containing up to 10 tyrosine residues potentially phosphorylated by the Src- or Syk-family kinases. Tyrosine-phosphorylated transmembrane adaptors bind (directly via SH2 domains or indirectly) other signaling molecules such as several cytoplasmic adaptors and enzymes. LAT is indispensable for TCR signaling (and participates also at signal transduction initiated by some other receptors), NTAL/LAB appears to play a LAT-like role in signaling initiated by BCR and some Fc-receptors; PAG/Cbp cooperates with Csk, the cytoplasmic tyrosine kinase negatively regulating Src-family kinases. Additional transmembrane adaptors exist (TRIM, SIT, LAX) that are however not palmitoylated and therefore excluded from the lipid rafts; structurally and functionally, the zeta-chain family proteins tightly associated with immunoreceptors and activating NK-receptors may be also considered as transmembrane adaptors.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Membrane microdomains; Lck; Csk; Signal transduction; Phosphorylation

1. Introduction

Plasma membrane is now considered to be a fluid mosaic of several types of dynamic microdomains distinguished by their lipid and protein composition. The best known type of such microdomains are so called membrane rafts of GEMs (glycosphingolipid-enriched microdomains). These are small areas of the membrane enriched in (glyco)sphingolipids, cholesterol and several specific types of extracellular transmembrane and cytoplasmic proteins (for reviews see [1–4]). Membrane rafts are, due to their specific lipid composition, exceptionally resistant to solubilization at low temperature by some common mild detergents (Triton X-100, NP-40, Brij-series, CHAPS), but soluble in alkyl-glycosidic detergents. Therefore, rafts/GEMs can be easily isolated by density gradient ultracentrifugation of membranes solubilized in suitable detergents, as they float to the position of the gradient corresponding to their low buoyant density (due to high lipid content). It should be noted that these detergent-resistant lipid rafts/GEMs are probably heterogeneous and several types of these complexes exist, differing in their lipid and protein composition [5,6]. Very little is known about other putative types of membrane microdomains that do not have the advantageous property of detergent insolubility.

The (glyco)lipids enriched in rafts possess mostly long, saturated fatty acid residues, in contrast to the non-raft membrane composed mainly of lipids containing fatty acids with multiple double bonds [7]. The alkyl chains of the raft lipids, together with suitable admixture of cholesterol, apparently form a more regularly arranged "liquid ordered phase", which may under physiological conditions at least transiently separate from the bulk membrane [8]. Most of typical transmembrane proteins are excluded from the rafts, an exception being a small subset of those possessing palmitic acid residue(s) attached covalently to cytoplasmic cysteine residue(s) close to the membrane-spanning domain. Other

* Tel.: +42-2-41729908; fax: +42-2-44472282.
E-mail address: horejsi@biomed.cas.cz (V. Hořejší).

0165-2478/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2003.10.013

proteins targeted to membrane rafts do not possess any transmembrane domains and are anchored in the membrane only by means of covalently attached saturated fatty acids or other aliphatic moieties. Among these are glycoproteins anchored in the exocytoplasmic leaflet of the raft membrane via glycosylphosphatidylinositol (GPI) [9] and several types of cytoplasmic proteins associated with the cytoplasmic membrane leaflet via double acylation (myristoylation and palmitoylation) or farnesylation and palmitoylation. The lipid-modified cytoplasmic proteins associated with lipid rafts are often important signaling molecules such as Src-family kinases [10–12], small and heterotrimeric G-proteins [13–15] or adaptor molecules, such as Raftlin [16], bringing other signaling proteins to the raft environment.

A number of transmembrane proteins, such as immunoreceptors [17–23] or certain cytokine receptors [24–26], become raft-associated following aggregation induced by interaction with their ligands. Some of the receptors are probably "peripherally" associated with membrane rafts even in the resting, non-ligated state [27–29]. Chemokine receptors and probably also some other G-protein-coupled receptors are constitutively associated with the raft microdomains [30,31].

The recent interest in leukocyte membrane rafts is due to their more and more generally accepted essential roles in signaling initiated through immunoreceptors such as TCR [17,18], BCR [19,20] and several Fc-receptors [21–23] but probably also several other receptors [24–26]. According to the simplest, currently popular model, these receptors are in their resting state devoid of any associated protein tyrosine kinases. Upon cross-linking by their natural or surrogate ligands (antibodies) their aggregates merge with membrane rafts and ITAM motifs present in cytoplasmic tails of their signaling chains (CD3, CD79, ζ-family proteins) become exposed to the Src kinases present in the rafts. Several other components of the earliest phases of immunoreceptor signaling such as transmembrane adaptors (see below) and the phospholipid phosphatidylinositol bisphosphate [32] reside also constitutively in membrane rafts.

As stated above, most of the few transmembrane proteins present constitutively in rafts are palmitoylated. Among these are e.g. TCR coreceptors CD4 and CD8 [33–36], pre-TCR [37], adhesion receptor CD44 [38] or proteolipid MAL [39], as well as a small group of transmembrane adaptor proteins LAT, PAG/Cbp, NTAL/LAB and probably a number of others [40].

These transmembrane adaptor proteins are the subject of the present review. Immunologically relevant cytoplasmic and transmembrane adaptors, including those associated with rafts, were recently reviewed in several other articles [41–47].

2. Linker for activation of T cells (LAT)

LAT was first described a decade ago as a phosphoprotein of 36–38 kDa (pp. 36–38) rapidly phosphorylated on tyrosine residues following TCR ligation [48]. Cloning of the gene revealed that the protein product is a type III (leaderless) transmembrane protein of 262 aa (long form) or 233 aa (short form) in humans, 242 aa in mouse, and 241 aa in rat [49,50]. The short human isoform arising by alternative splicing lacks residues 114–142 of the long form. Biological importance of this phenomenon is unclear. As a prototypic transmembrane adaptor protein, LAT possesses a short extracellular stretch of only 4 aa, a 24 aa transmembrane domain, and a cytoplasmic tail of 213–234 aa containing nine conserved tyrosine residues. The cytoplasmic tail of LAT also possesses a conserved juxtamembrane CxxC palmitoylation motif. Inhibition of palmitoylation prevents LAT association with rafts and inhibits its signaling functions [51–53], demonstrating the importance of localization to the unique environment of lipid rafts for its proper function. In agreement with this concept, a raft-targeted LAT/SLP-76 chimera restored signaling in LAT-deficient cells [54]. A soluble form of LAT lacking the transmembrane region was tyrosine-phosphorylated, recruited cytosolic signaling molecules and inhibited T cell activation, obviously due to sequestering signaling molecules from the rafts [55]. Interestingly, a significant fraction of LAT can be displaced from rafts by feeding T cells with polyunsaturated fatty acids (PUFA), obviously due to modification of membrane lipid composition and suppression of raft formation [56]; this has marked functional consequences (see below).

Following ligation of the TCR, LAT becomes rapidly phosphorylated by ZAP-70/Syk and associates with several key signaling molecules [49]. Some of them bind directly to LAT via SH2 domains (Grb2, Gads, Grap, PLCγ1, PI3-K, Cbl-b, 3BP2 and Shb), while others (SLP-76, Sos, Itk, Vav, Nck, SLAP-130, SKAP55) are associated indirectly via other adaptor molecules (i.e. Grb2, Gads, or Grap) [57]. It is likely that all these molecules do not associate simultaneously with a single LAT molecule but rather several or even multiple LAT-containing complexes exist. Formation of the multicomponent "signalosomes" assembled around the tyrosine-phosphorylated LAT is essential for propagation of the TCR-triggered signals. Therefore, mutant T cells lacking LAT (i.e. the deficient Jurkat variants, J.CaM2 and ANJ3) fail to mobilize Ca^{2+} and are unable to upregulate downstream markers of activation, such as IL-2 or CD69, upon TCR stimulation [52,58].

$LAT^{-/-}$ mice possess a normal population of B cells and both NK cells and platelets appear to function normally, but essentially lack mature peripheral T cells, because their thymocytes fail to develop beyond the double-negative (DN) stage [59].

Two recent studies described LAT knock-in mice in which the tyrosine residue (Y136) responsible for interaction with PLCγ1 was mutated to phenylalanine [60,61]. These mice had small thymi, similar to the $LAT^{-/-}$ mice, but showed only a partial block in thymocyte development, obviously due to compromised pre-TCR signaling. Curiously, during aging of these animals, a Th2-type $CD4^{+}$ population

expanded which led to an abnormal enlargement of the peripheral lymphoid organs and an infiltration of T cells into tissues. As expected, these T cells are unable to activate PLCγ1-calcium-dependent pathways.

Displacement of LAT from lipid rafts by PUFA treatment of T cells [56] appears to be the molecular mechanism by which PUFAs inhibit T cell signaling: although other signaling molecules (namely Lck) are also partially displaced from the rafts under these conditions, retaining LAT alone in lipid rafts effectively restores PLCγ1/calcium signaling in PUFA-treated T cells.

LAT was reported to associate with surface CD4 and CD8 coreceptors [62]; this association is based on the same coreceptor cysteine motif that mediates Lck binding. LAT competed with Lck for binding to individual coreceptor molecules but in contrast to Lck it preferentially associates with CD8 rather than CD4 in thymocytes. The authors suggested that LAT association with surface coreceptors is crucial for induction of its tyrosine phosphorylation and recruitment of downstream signaling mediators following co-engagement of the TCR with surface coreceptors. However, an alternative and possibly more likely alternative is that LAT functions mainly due to its mere presence in lipid rafts, irrespective of its direct association with the coreceptors.

In addition to its best explored roles in T cell activation, LAT is also tyrosine-phosphorylated upon stimulation of NK cells through FcγRIII and following direct contact with NK-sensitive target cells. This NK stimulation induces the association of LAT with several phosphotyrosine-containing proteins including PLCγ1. Overexpression of LAT in NK cells enhances antibody-dependent cell-mediated cytotoxicity and "natural cytotoxicity" [63]. Similarly, NK cell activation via CD2 cross-linking is accompanied by strong tyrosine phosphorylation of LAT, resulting in increased association with PI3-K and PLCγ1 [64]. Interestingly, in human NK cells, LAT is constitutively associated with 2B4 (CD244) and becomes tyrosine-phosphorylated and associated again with Grb2 and PLCγ following cross-linking of this receptor (it is not quite clear whether the LAT association with 2B4 is direct or rather mediated through lipid rafts). Therefore, CD244 may mediate NK cell triggering via a LAT-dependent signaling pathway [65–67].

Surprisingly, LAT expression in pre-B cells has been recently described [68]. LAT becomes tyrosine-phosphorylated upon cross-linking of the pre-B cell receptor. Transgenic mice which expressed LAT protein in B-lineage cells showed an increased proportion of pro- and large pre-B cells in the bone marrow and a remarkable reduction in the numbers of mature B cells in peripheral lymphoid tissues. The authors suggested that LAT may play a crucial role in the negative regulation of B cell development at the transition from pre-B to mature B cell stages, and that signal(s) via the pre-BCR may extinguish LAT expression, thus allowing pre-B cell differentiation towards the mature B cell stage.

LAT also plays important roles in activation of mast cells via FcεRI [44,69,70]. LAT is strongly tyrosine-phosphorylated after the receptor aggregation. Although LAT-deficient mice contain normal numbers of mast cells, LAT-deficient mice are resistant to IgE-mediated passive systemic anaphylaxis. LAT-deficient bone marrow-derived mast cells (BMMC) showed dramatic reduction in tyrosine phosphorylation of SLP-76, PLCγ1, and PLCγ2 and calcium mobilization. LAT-deficient BMMCs also exhibit defects in activation of MAPK, degranulation, and cytokine production after FcεRI cross-linking [70].

LAT is also involved in activation of platelets via integrin-associated protein (CD47) [71], FcγRIIa [72], collagen receptor GPVI and glycoprotein Ib (GPIb; receptor for von Willebrand factor) [73,74], however, the absence of LAT in the knock-out mice causes only partial defects in the platelet functions [74]; possibly another protein, such as NTAL (see below), may partially substitute for LAT in these animals.

3. Non-T cell activation linker (NTAL), linker for activation of B cells (LAB)

NTAL/LAB (to be called NTAL thereafter) [75,76] is structurally similar to LAT. It is the product of a previously cloned human gene of unclear function, *WBSCR5*. The polypeptide is 243 aa long and consists of a short (6 aa) extracellular peptide, a single transmembrane segment followed immediately by the CxxC palmitoylation motif and cytoplasmic domain containing 10 tyrosine motifs. Mouse NTAL is 40 aa shorter. Importantly, the genes encoding mouse LAT and NTAL share a strikingly similar exon–intron organization and thus are probably derived from a common ancestor. NTAL is expressed in an essentially complementary manner to LAT: it is found in B, NK and myeloid cells but not in resting T cells. Therefore, NTAL may be, as indicated by its names, a (partial) analog of LAT in B cells and myeloid cells. NTAL becomes tyrosine-phosphorylated by the Syk kinase (and also ubiquitinylated) following cross-linking of BCR, FcεRI and FcγRI, respectively, and then associates with other signaling molecules such as Grb2, Sos1, Gab1 and c-Cbl, but, in contrast to LAT, not with PLCγ. Thus, NTAL may be involved in activation of the Ras–Raf–MAPK signaling pathway but its importance in the PLCγ–PKC–Ca^{2+} pathway is less clear. Reduction of expression in B cells using RNA-interference leads to weaker BCR signaling [76] and expression of NTAL in LAT-negative T cells only partially rescues some aspects of the signaling process initiated through TCR, such as Erk activation and CD69 expression [75,76]. Furthermore, reconstitution of $LAT^{-/-}$ mice with NTAL (LAB) rescued partially thymic development but not full functionality of T cells.

It will be interesting to see whether NTAL plays a role also in signaling through other receptors and what is the relative importance of NTAL versus LAT in the cells expressing

both these molecules (NK cells, mast cells, platelets). These questions will be probably best approached by examining NTAL knock-out mice (presently under analysis).

4. Phosphoprotein associated with GEMs (PAG), Csk-binding protein (Cbp)

PAG/Cbp (hereafter referred to as PAG) is a 432 amino acid (aa) protein in humans and 429 aa in mouse, each containing a short extracellular domain (16–18 aa), a transmembrane domain (20 aa) followed by the CxxC palmitoylation motif, and a large cytoplasmic domain of 387–396 aa [77,78]. However, PAG migrates as a 75–80 kDa molecule in SDS PAGE, obviously because of its low isoelectric point and therefore atypically low SDS binding. PAG mRNA is detectable in most tissues, the expression being strongest in peripheral blood lymphocytes, placenta, heart, and lung. Interestingly, a recent study has demonstrated that while polyunsaturated fatty acid (PUFA) treatment of cells results in displacement of LAT and Lck from T cell rafts, PAG is unaffected [56]. This suggests that some structural feature other than palmitoylation is important for targeting and maintaining PAG in the rafts.

The cytoplasmic domain of PAG contains 10 tyrosine residues, nine of which are potential substrates for Src kinases [YxxV/I/L]. Indeed, Fyn or Lck but not Syk or ZAP70 can phosphorylate PAG [77,78] and Fyn was identified as the major PAG kinase in T cells in vivo [79]. Additionally, PAG contains multiple serine and threonine residues, which are potential sites for phosphorylation, as well as two proline rich regions. PAG can in vitro bind a number of signaling molecules via their SH2 domains, such as Syk, Lyn, Fyn, Shc, Grb2, SLP-76, ZAP70, PI3-K, Lck, Vav, GAP, and Csk. However, only two of these proteins, Fyn and Csk, are consistently found associated with PAG in vivo. Mutational analysis of individual tyrosine residues demonstrated that Csk binds (via its SH2 domain) to Y317 in human PAG (but Y299 probably also contributes to this interaction). In contrast, the interaction with Fyn appears to be tyrosine-independent [77,78]. This corresponds to the previous finding that Csk mutants lacking the SH2 domain fail to translocate to the plasma membrane and also fail to regulate T cell receptor signaling [80].

The identification of PAG as a membrane adaptor for Csk provided a plausible mechanism by which this ubiquitous cytosolic kinase, the negative regulator of Src-family kinases, can be effectively targeted to its membrane-associated substrates. In resting αβT cells, PAG is the most prominently phosphorylated molecule; immediately upon TCR-triggered activation, PAG becomes dephosphorylated and the association with Csk is dramatically reduced [77,81,82]. This obviously helps to get rid of the Csk-mediated inhibition of Lck and Fyn during the initial phase of T cell activation. The results of an elegant recent study indicate that the phosphatase responsible in vivo for this activation-linked PAG dephosphorylation is probably CD45 [83]. It should be noted that Src-family kinases phosphorylate PAG, leading to Csk recruitment, which in turn inhibits them; thus, the PAG–Csk–Src-kinase–CD45 system is an interesting case of a negative feedback regulation. The activation of Csk following binding to PAG is not only due to the "topological" effect (approximation to the membrane-associated substrates) but probably also due to an allosteric enhancement in the complex [84]. This regulatory system is further fine-tuned by an interaction with PKA (regulated via cAMP) which increases the activity of Csk within the PAG–Csk complex and thus contributes to inhibition of TCR signaling [85].

PAG acts as a negative regulator of immunoreceptor signaling also in mast cells. However, in contrast to αβT cells, aggregation of the IgE receptor, FcεRI, is accompanied by rapid phosphorylation of PAG and increased Csk recruitment, which obviously serves to suppress activity of Lyn kinase and thereby mitigate the intensity of signaling. Similarly as in T cells, overexpression of PAG inhibits receptor signaling also in mast cells [86]. Cross-linking of BCR in B cells also induces an increase in PAG phosphorylation [87]; this is in marked contrast to the αβT cells and probably reflects the fact that Src kinases in B cells are more involved in negative regulation of the antigen receptor signaling (phosphorylation of ITIM motifs in negative regulators such as CD22). Disturbances of the PAG–Csk system appear to contribute substantially to leukaemic-like transformation of cattle lymphocytes by the protozoal parasite *Theileria* [88]. The parasite is apparently able to suppress expression of PAG, resulting in increased kinase activity of Hck involved in proliferation and leukaemic-like phenotype of the infected cells. Killing of the intracellular parasite by an affective chemotherapy results in rapid reexpression of tyrosine-phosphorylated PAG, membrane (raft) recruitment of Csk and suppression of Hck activity which is probably at least partially responsible for the loss of the transformed phenotype.

PAG is able to interact via its C-terminal VTRL motif with a PDZ domain of the cytoplasmic adaptor protein EBP50 [81,89]. EBP50 in turn interacts with ezrin-radixin-moesin (ERM) proteins, thus providing a link between lipid rafts and the actin cytoskeleton. As shown in one of the studies, upon T cell activation this association is lost allowing the rafts to migrate to the immune synapse. Overexpression of PAG reduces raft mobility and inhibits immune synapse formation [81]. This may be another mechanism by which PAG can regulate T cell activation.

5. Other transmembrane adaptors

There are several other transmembrane adaptor proteins, i.e. molecules consisting of a very short extracellular peptide, single transmembrane helix and intracellular domain containing multiple tyrosine-based motifs. Formally, the zeta-chain of the TCR complex is such a protein, as well as

the other zeta-chain family proteins (η, DAP-12, DAP-10) closely associated with TCR, some Fc-receptors and with some activating NK cell and myeloid cell receptors. Three other transmembrane adaptors associated with membrane receptors either weakly or not at all are TRIM [90], SIT [91] and LAX [92]. These proteins are not associated with membrane rafts, do not possess the palmitoylation motif and all of them appear to be involved in some aspects of regulation of immunoreceptor signaling.

Systematic searching of available sequence databases indicates potential existence of several other transmembrane adaptor molecules, some of them possessing a palmitoylation motif and therefore probably associated with membrane rafts (unpublished data from our laboratory). One of them, expressed mainly in T cells and named LIME (standing for Lck-interacting membrane protein), becomes tyrosine-phosphorylated following CD4 or CD8 cross-linking. Phosphorylated LIME binds Lck and Csk and may be involved in some aspects of coreceptor signaling [93,94].

Acknowledgements

This work was supported from the project Center of Molecular and Cellular Immunology (LN00A026) of the Czech Ministry of Education, Youth and Sports.

References

[1] Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997;387:569–72.
[2] Harder T. Raft membrane domains and immunoreceptor functions. Adv Immunol 2001;77:45–92.
[3] Cherukuri A, Dykstra M, Pierce SK. Floating the raft hypothesis: lipid rafts play a role in immune cell activation. Immunity 2001;14:657–60.
[4] Horejsi V. The roles of membrane microdomains (rafts) in T cell activation. Immunol Rev 2003;191:148–64.
[5] Gomez-Mouton C, Abad JL, Mira E, et al. Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. Proc Natl Acad Sci USA 2001;98:9642–7.
[6] Schade AE, Levine AD. Lipid raft heterogeneity in human peripheral blood T lymphoblasts: a mechanism for regulating the initiation of TCR signal transduction. J Immunol 2002;168:2233–9.
[7] Brown DA, London E. Structure and origin of ordered lipid domains in biological membranes. J Membr Biol 1998;164:103–14.
[8] Schroeder R, London E, Brown D. 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 1994;91:12130–4.
[9] Horejsi V, Cebecauer M, Cerny J, Brdicka T, Angelisova P, Drbal K. Signal transduction in leucocytes via GPI-anchored proteins: an experimental artefact or an aspect of immunoreceptor function? Immunol Lett 1998;63:63–73.
[10] Cinek T, Horejsi V. The nature of large noncovalent complexes containing glycosyl- phosphatidylinositol-anchored membrane glycoproteins and protein tyrosine kinases. J Immunol 1992;149:2262–70.
[11] Dráberová L, Dráber P. Thy-1 glycoprotein and src-like protein-tyrosine kinase p53/p56lyn are associated in large detergent-resistant complexes in rat basophilic leukemia cells. Proc Natl Acad Sci USA 1993;90:3611–5.
[12] Stefanova I, Horejsi V, Ansotegui IJ, Knapp W, Stockinger H. GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. Science 1991;254:1016–9.
[13] Prior IA, Harding A, Yan J, Sluimer J, Parton RG, Hancock JF. GTP-dependent segregation of H-ras from lipid rafts is required for biological activity. Nat Cell Biol 2001;3:368–75.
[14] Parolini I, Sargiacomo M, Lisanti MP, Peschle C. Signal transduction and glycophosphatidylinositol-linked proteins (lyn, lck, CD4, CD45, G proteins, and CD55) selectively localize in Triton- insoluble plasma membrane domains of human leukemic cell lines and normal granulocytes. Blood 1996;87:3783–94.
[15] Oh P, Schnitzer JE. Segregation of heterotrimeric G proteins in cell surface microdomains. Gq binds caveolin to concentrate in caveolae, whereas Gi and Gs target lipid rafts by default. Mol Biol Cell 2001;12:685–98.
[16] Saeki K, Miura Y, Aki D, Kurosaki T, Yoshimura A. The B cell-specific major raft protein, Raftlin, is necessary for the integrity of lipid raft and BCR signal transduction. EMBO J 2003;22:3015–26.
[17] Xavier R, Brennan T, Li Q, McCormack C, Seed B. Membrane compartmentation is required for efficient T cell activation. Immunity 1998;8:723–32.
[18] Montixi C, Langlet C, Bernard AM, et al. Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. EMBO J 1998;17:5334–48.
[19] Cheng PC, Dykstra ML, Mitchell RN, Pierce SK. A role for lipid rafts in B cell antigen receptor signaling and antigen targeting. J Exp Med 1999;190:1549–60.
[20] Pierce SK. Lipid rafts and B-cell activation. Nat Rev Immunol 2002;2:96–105.
[21] Field KA, Holowka D, Baird B. FceRI-mediated recruitment of p53/56lyn to detergent-resistant membrane domains accompanies cellular signaling. Proc Natl Acad Sci USA 1995;92:9201–5.
[22] Lang ML, Chen YW, Shen L, Gao H, Lang GA, Wade TK, et al. IgA Fc receptor (FcaR) cross-linking recruits tyrosine kinases, phosphoinositide kinases and serine/threonine kinases to glycolipid rafts. Biochem J 2002;364:517–25.
[23] Kono H, Suzuki T, Yamamoto K, Okada M, Yamamoto T, Honda Z. Spatial raft coalescence represents an initial step in FcgR signaling. J Immunol 2002;169:193–203.
[24] Sehgal PB, Guo GG, Shah M, Kumar V, Patel K. Cytokine signaling: STATS in plasma membrane rafts. J Biol Chem 2002;277:12067–74.
[25] Vereb G, Matko J, Vamosi G, Ibrahim SM, Magyar E, Varga S, et al. Cholesterol-dependent clustering of IL-2Ra and its colocalization with HLA and CD48 on T lymphoma cells suggest their functional association with lipid rafts. Proc Natl Acad Sci USA 2000;97:6013–8.
[26] Subramaniam PS, Johnson HM. Lipid microdomains are required sites for the selective endocytosis and nuclear translocation of IFN-g, its receptor chain IFN-g receptor-1, and the phosphorylation and nuclear translocation of STAT1a. J Immunol 2002;169:1959–69.
[27] Drevot P, Langlet C, Guo XJ, Bernard AM, Colard O, Chauvin JP, et al. TCR signal initiation machinery is pre-assembled and activated in a subset of membrane rafts. EMBO J 2002;21:1899–908.
[28] Pham LV, Tamayo AT, Yoshimura LC, Lo P, Terry N, Reid PS, et al. A CD40 Signalosome anchored in lipid rafts leads to constitutive activation of NF-kB and autonomous cell growth in B cell lymphomas. Immunity 2002;16:37–50.
[29] Cottin V, Doan JE, Riches DW. Restricted localization of the TNF receptor CD120a to lipid rafts: a novel role for the death domain. J Immunol 2002;168:4095–102.
[30] Manes S, Lacalle RA, Gomez-Mouton C, del Real G, Mira E, Martinez AC. Membrane raft microdomains in chemokine receptor function. Semin Immunol 2001;13:147–57.
[31] Popik W, Alce TM, Au WC. Human immunodeficiency virus type 1 uses lipid raft-colocalized CD4 and chemokine receptors

for productive entry into CD4+ T cells. J Virol 2002;76:4709–22.
[32] Hope HR, Pike LJ. Phosphoinositides and phosphoinositide-utilizing enzymes in detergent- insoluble lipid domains. Mol Biol Cell 1996;7:843–51.
[33] Cinek T, Hilgert I, Horejsi V. An alternative way of CD4 and CD8 association with protein kinases of the Src family. Immunogenetics 1995;41:110–6.
[34] Parolini I, Topa S, Sorice M, Pace A, Ceddia P, Montesoro E, et al. Phorbol ester-induced disruption of the CD4-Lck complex occurs within a detergent-resistant microdomain of the plasma membrane. Involvement of the translocation of activated protein kinase C isoforms. J Biol Chem 1999;274:14176–87.
[35] Arcaro A, Gregoire C, Bakker TR, Baldi L, Jordan M, Goffin L, et al. CD8b endows CD8 with efficient coreceptor function by coupling T cell receptor/CD3 to raft-associated CD8/p56lck complexes. J Exp Med 2001;194:1485–95.
[36] Millan J, Cerny J, Horejsi V, Alonso MA. CD4 segregates into specific detergent-resistant T-cell membrane microdomains. Tissue Antigens 1999;53:33–40.
[37] Saint-Ruf C, Panigada M, Azogui O, Debey P, von Boehmer H, Grassi F. Different initiation of pre-TCR and gdTCR signalling. Nature 2000;406:524–7.
[38] Neame SJ, Uff CR, Sheikh H, Wheatley SC, Isacke CM. CD44 exhibits a cell type dependent interaction with Triton X-100 insoluble, lipid rich, plasma membrane domains. J Cell Sci 1995;108:3127–35.
[39] Millan J, Alonso MA. MAL, a novel integral membrane protein of human T lymphocytes, associates with glycosylphosphatidylinositol-anchored proteins and Src- like tyrosine kinases. Eur J Immunol 1998;28:3675–84.
[40] Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci USA 2003;100:5813–8.
[41] Lindquist JA, Simeoni L, Schraven B. Transmembrane adapters: attractants for cytoplasmic effectors. Immunol Rev 2003;191:165–82.
[42] Jordan MS, Singer AL, Koretzky GA. Adaptors as central mediators of signal transduction in immune cells. Nat Immunol 2003;4:110–6.
[43] Burack WR, Cheng AM, Shaw AS. Scaffolds, adaptors and linkers of TCR signaling: theory and practice. Curr Opin Immunol 2002;14:312–6.
[44] Rivera J. Molecular adapters in FceRI signaling and the allergic response. Curr Opin Immunol 2002;14:688–93.
[45] Leo A, Wienands J, Baier G, Horejsi V, Schraven B. Adapters in lymphocyte signaling. J Clin Invest 2002;109:301–9.
[46] Leo A, Schraven B. Adapters in lymphocyte signalling. Curr Opin Immunol 2001;13:307–16.
[47] Veillette A, Latour S, Davidson D. Negative regulation of immunoreceptor signaling. Annu Rev Immunol 2002;20:669–707.
[48] Sieh M, Batzer A, Schlessinger J, Weiss A. GRB2 and phospholipase C-g 1 associate with a 36- to 38-kilodalton phosphotyrosine protein after T-cell receptor stimulation. Mol Cell Biol 1994;14:4435–42.
[49] Zhang W, Sloan-Lancaster J, Kitchen J, Trible RP, Samelson LE. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. Cell 1998;92:83–92.
[50] Weber JR, Orstavik S, Torgersen KM, Danbolt NC, Berg SF, Ryan JC, et al. Molecular cloning of the cDNA encoding pp36, a tyrosine-phosphorylated adaptor protein selectively expressed by T cells and natural killer cells. J Exp Med 1998;187:1157–61.
[51] Zhang W, Trible RP, Samelson LE. LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity 1998;9:239–46.
[52] Lin J, Weiss A, Finco TS. Localization of LAT in glycolipid-enriched microdomains is required for T cell activation. J Biol Chem 1999;274:28861–4.
[53] Webb Y, Hermida-Matsumoto L, Resh MD. Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. J Biol Chem 2000;275:261–70.
[54] Boerth NJ, Sadler JJ, Bauer DE, Clements JL, Gheith SM, Koretzky GA. Recruitment of SLP-76 to the membrane and glycolipid-enriched membrane microdomains replaces the requirement for linker for activation of T cells in T cell receptor signaling. J Exp Med 2000;192:1047–58.
[55] Torgersen KM, Vaage JT, Rolstad B, Tasken K. A soluble LAT deletion mutant inhibits T-cell activation: reduced recruitment of signalling molecules to glycolipid-enriched microdomains. Cell Signal 2001;13:213–20.
[56] Zeyda M, Staffler G, Horejsi V, Waldhausl W, Stulnig TM. LAT displacement from lipid rafts as a molecular mechanism for the inhibition of T cell signaling by polyunsaturated fatty acids. J Biol Chem 2002;277:28418–23.
[57] Paz PE, Wang S, Clarke H, Lu X, Stokoe D, Abo A. Mapping the Zap-70 phosphorylation sites on LAT (linker for activation of T cells) required for recruitment and activation of signalling proteins in T cells. Biochem J 2001;356:461–71.
[58] Zhang W, Irvin BJ, Trible RP, Abraham RT, Samelson LE. Functional analysis of LAT in TCR-mediated signaling pathways using a LAT-deficient Jurkat cell line. Int Immunol 1999;11:943–50.
[59] Zhang W, Sommers CL, Burshtyn DN, Stebbins CC, DeJarnette JB, Trible RP, et al. Essential role of LAT in T cell development. Immunity 1999;10:323–32.
[60] Aguado E, Richelme S, Nunez-Cruz S, Miazek A, Mura AM, Richelme M, et al. Induction of T helper type 2 immunity by a point mutation in the LAT adaptor. Science 2002;296:2036–40.
[61] Sommers CL, Park CS, Lee J, Feng C, Fuller CL, Grinberg A, et al. A LAT mutation that inhibits T cell development yet induces lymphoproliferation. Science 2002;296:2040–3.
[62] Bosselut R, Zhang W, Ashe JM, Kopacz JL, Samelson LE, Singer A. Association of the adaptor molecule LAT with CD4 and CD8 coreceptors identifies a new coreceptor function in T cell receptor signal transduction. J Exp Med 1999;190:1517–26.
[63] Jevremovic D, Billadeau DD, Schoon RA, et al. Cutting edge: a role for the adaptor protein LAT in human NK cell-mediated cytotoxicity. J Immunol 1999;162:2453–6.
[64] Inoue H, Miyaji M, Kosugi A, Nagafuku M, Okazaki T, Mimori T, et al. Lipid rafts as the signaling scaffold for NK cell activation: tyrosine phosphorylation and association of LAT with phosphatidylinositol 3-kinase and phospholipase C-g following CD2 stimulation. Eur J Immunol 2002;32:2188–98.
[65] Klem J, Verrett PC, Kumar V, Schatzle JD. 2B4 is constitutively associated with linker for the activation of T cells in glycolipid-enriched microdomains: properties required for 2B4 lytic function. J Immunol 2002;169:55–62.
[66] Bottino C, Augugliaro R, Castriconi R, Nanni M, Biassoni R, Moretta L, et al. Analysis of the molecular mechanism involved in 2B4-mediated NK cell activation: evidence that human 2B4 is physically and functionally associated with the linker for activation of T cells. Eur J Immunol 2000;30:3718–22.
[67] Chuang SS, Kumaresan PR, Mathew PA. 2B4 (CD244)-mediated activation of cytotoxicity and IFN-g release in human NK cells involves distinct pathways. J Immunol 2001;167:6210–6.
[68] Oya K, Wang J, Watanabe Y, Koga R, Watanabe T. Appearance of the LAT protein at an early stage of B-cell development and its possible role. Immunology 2003;109:351–9.
[69] Kimura T, Hisano M, Inoue Y, Adachi M. Tyrosine phosphorylation of the linker for activator of T cells in mast cells by stimulation with the high affinity IgE receptor. Immunol Lett 2001;75:123–9.
[70] Saitoh S, Arudchandran R, Manetz TS, Zhang W, Sommers CL, Love PE, et al. LAT is essential for FceRI-mediated mast cell activation. Immunity 2000;12:525–35.
[71] Trumel C, Plantavid M, Levy-Toledano S, Ragab A, Caen JP, Aguado E, et al. Platelet aggregation induced by the C-terminal peptide of

thrombospondin-1 requires the docking protein LAT but is largely independent of aIIb/b3. J Thromb Haemost 2003;1:320–9.
[72] Ragab A, Bodin S, Viala C, Chap H, Payrastre B, Ragab-Thomas J. The tyrosine phosphatase 1B regulates LAT phosphorylation and platelet aggregation upon FcgRIIa cross-linking. J Biol Chem 2003;278:40923–32.
[73] Asazuma N, Wilde JI, Berlanga O, Leduc M, Leo A, Schweighoffer E, et al. Interaction of linker for activation of T cells with multiple adapter proteins in platelets activated by the glycoprotein VI-selective ligand, convulxin. J Biol Chem 2000;275:33427–34.
[74] Pasquet JM, Gross B, Quek L, Asazuma N, Zhang W, Sommers CL, et al. LAT is required for tyrosine phosphorylation of phospholipase Cg2 and platelet activation by the collagen receptor GPVI. Mol Cell Biol 1999;19:8326–34.
[75] Brdicka T, Imrich M, Angelisova P, Brdickova N, Horvath O, Spicka J, et al. Non-T cell activation linker (NTAL): a transmembrane adaptor protein involved in immunoreceptor signaling. J Exp Med 2002;196:1617–26.
[76] Janssen E, Zhu M, Zhang W, Koonpaew S, Zhang W. LAB: a new membrane-associated adaptor molecule in B cell activation. Nat Immunol 2003;4:117–23.
[77] Brdicka T, Pavlistova D, Leo A, Bruyns E, Korinek V, Angelisova P, et al. Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase Csk and is involved in regulation of T cell activation. J Exp Med 2000;191:1591–604.
[78] Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K, et al. Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. Nature 2000;404:999–1003.
[79] Yasuda K, Nagafuku M, Shima T, Okada M, Yagi T, Yamada T, et al. Cutting edge: Fyn is essential for tyrosine phosphorylation of Csk-binding protein/phosphoprotein associated with glycolipid-enriched microdomains in lipid rafts in resting T cells. J Immunol 2002;169:2813–7.
[80] Cloutier JF, Chow LM, Veillette A. Requirement of the SH3 and SH2 domains for the inhibitory function of tyrosine protein kinase p50csk in T lymphocytes. Mol Cell Biol 1995;15:5937–44.
[81] Itoh K, Sakakibara M, Yamasaki S, Takeuchi A, Arase H, Miyazaki M, et al. Cutting edge: negative regulation of immune synapse formation by anchoring lipid raft to cytoskeleton through Cbp-EBP50-ERM assembly. J Immunol 2002;168:541–4.
[82] Torgersen KM, Vang T, Abrahamsen H, Yaqub S, Horejsi V, Schraven B, et al. Release from tonic inhibition of T cell activation through transient displacement of C-terminal Src kinase (Csk) from lipid rafts. J Biol Chem 2001;276:29313–8.
[83] Davidson D, Bakinowski M, Thomas ML, Horejsi V, Veillette A. Phosphorylation-dependent regulation of T-cell activation by PAG/Cbp, a lipid raft-associated transmembrane adaptor. Mol Cell Biol 2003;23:2017–28.
[84] Takeuchi S, Takayama Y, Ogawa A, Tamura K, Okada M. Transmembrane phosphoprotein cbp positively regulates the activity of the carboxyl-terminal src kinase. Csk J Biol Chem 2000;275:29183–6.
[85] Vang T, Abrahamsen H, Myklebust S, Horejsi V, Tasken K. Combined spatial and enzymatic regulation of Csk by cAMP and protein kinase a inhibits T cell receptor signaling. J Biol Chem 2003;278:17597–600.
[86] Ohtake H, Ichikawa N, Okada M, Yamashita T. Cutting Edge: Transmembrane phosphoprotein Csk-binding protein/phosphoprotein associated with glycosphingolipid-enriched microdomains as a negative feedback regulator of mast cell signaling through the FceRI. J Immunol 2002;168:2087–90.
[87] Awasthi-Kalia M, Schnetkamp PP, Deans JP. Differential effects of filipin and methyl-b-cyclodextrin on B cell receptor signaling. Biochem Biophys Res Commun 2001;287:77–82.
[88] Baumgartner M, Angelisova P, Setterblad N, Mooney N, Werling D, Horejsi V, et al. Constitutive exclusion of Csk from Hck-positive membrane microdomains permits Src kinase-dependent proliferation of Theileria-transformed B lymphocytes. Blood 2003;101:1874–81.
[89] Brdickova N, Brdicka T, Andera L, Spicka J, Angelisova P, Milgram SL, et al. Interaction between two adapter proteins, PAG and EBP50: a possible link between membrane rafts and actin cytoskeleton. FEBS Lett 2001;507:133–6.
[90] Bruyns E, Kirchgessner H, Meuer S, Schraven B. Biochemical analysis of the CD45-p56lck complex in Jurkat cells lacking expression of lymphocyte phosphatase-associated protein. Int Immunol 1998;10:185–94.
[91] Marie-Cardine A, Kirchgessner H, Bruyns E, Shevchenko A, Mann M, Autschbach F, et al. SHP2-interacting transmembrane adaptor protein (SIT), a novel disulfide- linked dimer regulating human T cell activation. J Exp Med 1999;189:1181–94.
[92] Zhu M, Janssen E, Leung K, Zhang W. Molecular cloning of a novel gene encoding a membrane-associated adaptor protein (LAX) in lymphocyte signaling. J Biol Chem 2002;277:46151–8.
[93] Brdickova N, Brdicka T, Angelisova P, Horvath O, Spicka J, Hilgert I, et al. LIME: a new membrane Raft-associated adaptor protein involved in CD4 and CD8 coreceptor signaling. J Exp Med 2003;198:1453–62.
[94] Hur EM, Son M, Lee OH, Choi YB, Park C, Lee H, et al. LIME, a novel transmembrane adaptor protein, associates with p56lck and mediates T cell activation. J Exp Med 2003;198:1463–73.