58 Smith, K.G., Strasser, A. and Vaux, D.L. (1996) *EMBO J.* 15, 5167–5176
59 Sarin, A., Williams, M.S., Alexander-Miller, M.A., Berzofsky, J.A., Zacharchuk, C.M. and Henkart, P.A. (1997) *Immunity* 6, 209–215
60 Trapani, J.A., Jans, D.A., Jans, P.J., Smyth, M.J., Browne, K.A. and Sutton, V.R. (1998) *J. Biol. Chem.* 273, 27934–27938
61 Sarin, A., Haddad, E.K. and Henkart, P.A. (1998) *J. Immunol.* 161, 2810–2816
62 MacDonald, G., Shi, L., Vande Velde, C., Lieberman, J. and Greenberg, A.H. (1999) *J. Exp. Med.* 189, 131–144
63 Vucic, D., Kaiser, W.J., Harvey, A.J. and Miller, L.K. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 10183–10188
64 Seshagiri, S. and Miller, L.K. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 13606–13611
65 Yew, P.R. and Berk, A.J. (1992) *Nature* 357, 82–85
66 Huang, D.C., Cory, S. and Strasser, A.S. (1997) *Oncogene* 14, 405–414
67 Dahl, J., Jurczak, A., Cheng, L.A., Baker, D.C. and Benjamin, T.L. (1998) *J. Virol.* 72, 3221–3226
68 Liu, J.L., Ye, Y., Lee, L.F. and Kung, H.J. (1998) *J. Virol.* 72, 388–395
69 Zimring, J.C., Goodbourn, S. and Offermann, M.K. (1998) *J. Virol.* 72, 701–707
70 Shisler, J.L., Senkevich, T.G., Berry, M.J. and Moss, B. (1998) *Science* 279, 102–105
71 Ayyavoo, V., Mahboubi, A., Mahalingam, S. *et al.* (1997) *Nat. Med.* 3, 1117–1123

GPI-microdomains: a role in signalling via immunoreceptors

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Glycosylphosphatidylinositol (GPI)-anchored proteins and glycosphingolipids are assembled on the leukocyte surface within membrane microdomains, which also accommodate a set of cytoplasmic signalling molecules (Src family kinases, G-proteins, linker proteins). Recent results suggest that these membrane specializations mediate not only signal transduction via GPI-proteins and glycolipids but also play important roles in initiation of signalling via immunoreceptors.

Several leukocyte surface proteins are anchored in the membrane via the glycolipid glycosylphosphatidylinositol (GPI)[1] (Fig. 1). A striking feature of these structurally diverse proteins (see Box 1) is that their ligation on the cell surface by suitable antibodies results in signal transduction that is characterized by: (1) transient elevation of cytoplasmic $[Ca^{2+}]$; (2) tyrosine phosphorylation of cellular substrates; (3) initiation of effector functions such as oxidative burst or degranulation in granulocytes; and (4) even triggering of T-cell proliferation and functional differentiation into effector cells[2,3]. Such signalling capacity is surprising considering that these molecules have no transmembrane and intracellular moieties and thus no direct contact with the cell interior. Similarly, crosslinking of some glycolipids by antibodies also elicits signal transduction and cellular responses[4,5].

GPI-complexes and membrane microdomains

Another characteristic feature of the GPI-anchored proteins is that, following membrane solubilization by most types of mild detergents at low temperature, they are found in large detergent-insoluble complexes enriched in GPI-anchored proteins, glycosphingolipids, cholesterol, Src family protein tyrosine-kinases (PTKs) and G-proteins but devoid of most transmembrane proteins[6–10]. These 'GPI-complexes' [also called glycosphingolipid-cholesterol rafts, detergent-insoluble glycolipid-enriched domains (DIGs)[11] or glycosphingolipid-enriched membrane domains (GEMs)[12]] are of low buoyant density under the conditions of density gradient ultracentrifugation. The detergent-resistant GPI-complexes seem to correspond to membrane microdomains of distinct composition, different from the rest of the membrane. The GPI-microdomains can be viewed, with some simplification, as small semi-liquid islands floating in the more liquid phospholipid-rich bulk of the leukocyte membrane (Fig. 2).

Structures similar to leukocyte GPI-microdomains seem to exist in many, perhaps most, cell types and have been thoroughly studied especially in polarized epithelial and endothelial cells (reviewed in Ref. 11). In these cells, the GPI-microdomains (rafts, DIGs) can fuse to form much larger membrane sheets and comprise a considerable part of the apical surface of these cells although being largely absent

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PII: S0167-5699(99)01489-9

from the basolateral surface. In many cell types (but not leukocytes), the GPI-microdomains appear to be closely linked to (but distinct from) so called caveoli, flask-shaped membrane invaginations probably involved in a specific sort of endocytosis as well as receptor signalling[13,14]. The relationship between caveoli and GPI-microdomains (membrane rafts) has been controversial. At present a consensus exists that these are different entities sharing some (high cholesterol content, resistance to detergent solubilization, low buoyant density) but differing in other features (presence versus absence of the cholesterol-binding protein caveolin, overall protein composition, morphological characteristics). GPI-domains and caveoli can fuse under certain experimental conditions[14,15].

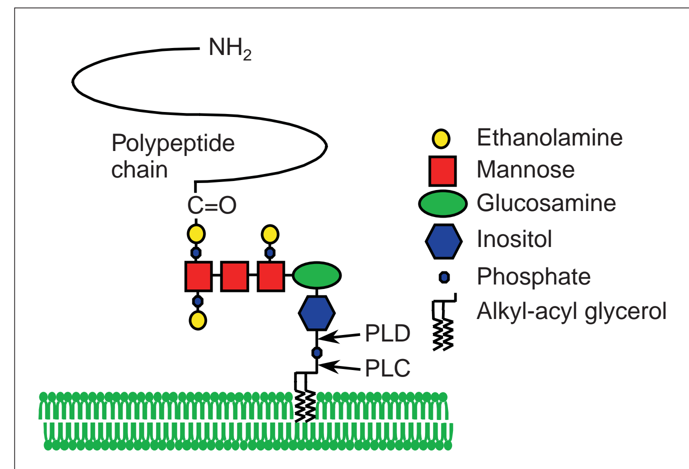

Fig. 1. *Schematic representation of a glycosylphosphatidylinositol (GPI)-anchored protein. The C-terminal amino acid of the polypeptide chain is covalently bound to the glycolipid via an amide bond to ethanolamine residue. Structural variants of the glycan part exist in different cells and species. The sites cleaved by phospholipase C (PLC) and phospholipase D (PLD) are shown.*

Actual or artefact?

The very existence of the GPI-microdomains has often been questioned because they could be detergent artefacts: either simply clusters of the membrane components exhibiting low temperature affinity toward detergent molecules or, alternatively, remnants of the originally homogeneous membrane from which the detergent has etched-out most of the membrane components (phosphoglycerolipids, most transmembrane proteins) and the poorly soluble remains aggregated together. Several lines of evidence seem to rule out the possibility that the detergent-resistant GPI-complexes are detergent solubilization artefacts[16–20]. However, the question of how precisely the size and composition of the native membrane microdomains correspond to that of the detergent-insoluble complexes requires further study (see below).

The integrity of the GPI-microdomains seems to be maintained in part due to intrinsic affinity of two of their major lipidic components – cholesterol and glycosphingolipids. These structures are probably formed biosynthetically as a specific species of secretory vesicles leaving the Golgi apparatus and fusing with plasma membrane[11]. An important factor might be the length and saturated nature of the fatty acid residues present in the glycosphingolipids and GPI-anchors that result in tighter packing and thus lower mobility (fluidity) of these areas of the membrane[18,19]. The microdomains thus appear to be areas of 'ordered liquid phase' in the membrane[19]. The cytoplasmic protein components of these microdomains (Src family PTKs, trimeric G-proteins) are also associated with the specific lipid environment of these microdomains via saturated fatty acids, myristic acid and palmitic acid, covalently bound at the N-termini of these proteins[12,21,22]. The GPI-anchored proteins might effectively be just a special sort of glycolipid with an affinity for this membrane subcompartment (because of their overall physicochemical similarity to glycosphingolipids).

GPI-microdomains obviously contain such prominent signalling molecules as Src family protein tyrosine kinases (PTK) and G-proteins. This seems to explain the striking signalling capacity of GPI-anchored proteins and glycolipids. Crosslinking of these molecules

Box 1. Some GPI-anchored leukocyte surface proteins

Name	Function
CD14 [lipopolysaccharide (LPS) receptor]	Receptor for the bacterial cell wall component LPS
CD16b [Fcγ receptor type 3 (FcγRIII)]	IgG receptor
CD90 (Thy-1)	Probably adhesion molecule
CD58 (LFA-3)	Adhesion molecule
CD48	Adhesion molecule
CD55 (decay accelerating factor)	Complement-protecting protein
CD59	Complement-protecting protein
CD73 (5′ nucleotidase)	Ectoenzyme
CD157 (ADP-ribosyl cyclase)	Ectoenzyme
RT-6 (arginine ADP-ribosyl transferase)	Ectoenzyme
CD87 [urokinase-type plasminogen activator receptor (uPA-R)]	Protease receptor
Ly-6 family proteins	Function unclear
CD24 [heat stable antigen (HSA)]	Function unclear (proteoglycan)
CD52	Function unclear (proteoglycan)

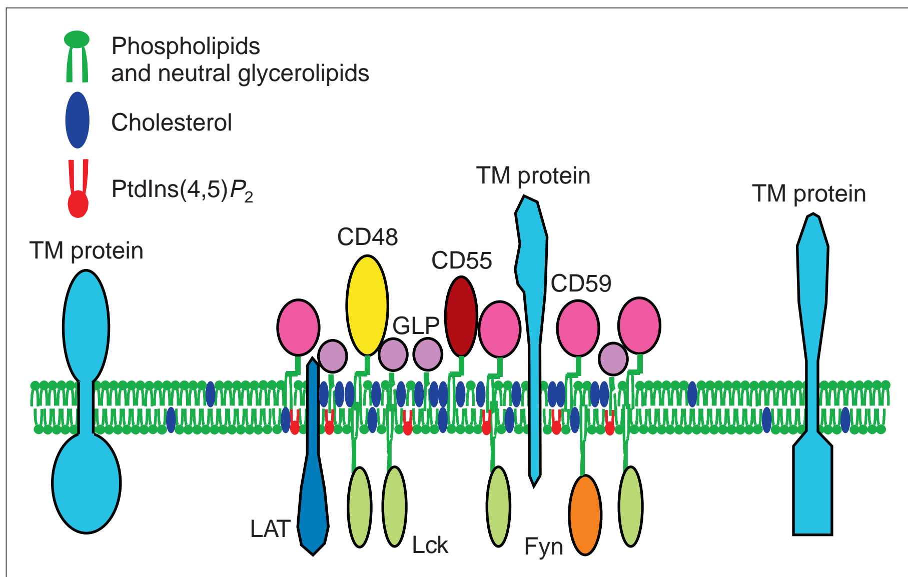

Fig. 2. *Hypothetical structure of a T-cell surface glycosylphosphatidylinositol (GPI) microdomain. These areas of the membrane are distinguished by high content of GLP, cholesterol, GPI-anchored proteins (e.g. CD59, CD55, CD48), Src family kinases (Lck, Fyn), some other signalling proteins (e.g. LAT) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. Only exceptional TM proteins are present in these microdomains. Abbreviations: GLP, glycosphingolipids; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; TM, transmembrane.*

within the external part of the GPI-microdomains by multivalent antibodies (and possibly also by natural ligands) can cause simultaneous redistribution (approximation) of the Src family PTKs at their cytoplasmic side, initiating autophosphorylation and activation of the PTKs, followed by phosphorylation of their protein substrates and thus triggering of signalling cascades (Fig. 3). It is important to note that the (predominantly saturated) fatty acid residues present in GPI-anchors and glycosphingolipids are long enough to penetrate into the opposing cytoplasmic leaflet of the membrane and directly interact with aliphatic chains of the cytoplasmic signalling molecules. This might be the mechanistic factor responsible for co-redistribution of extracellular (GPI-proteins, glycolipids) and cytoplasmic molecules (Src family kinases).

Signalling via GPI-anchored proteins has mostly been achieved in an artificial way – by antibody crosslinking[3]. Examples of GPI-anchored receptors that signal upon interaction with their natural ligands include CD87 [urokinase-type plasminogen activator receptor (uPA-R)][23], CD16b (neutrophil IgG receptor)[24] and CD14 [lipopolysaccharide (LPS) receptor][25].

A role for the GPI-microdomains in immunoreceptor signalling?

There is evidence from recent studies that GPI-microdomains might play not only a role in signalling via GPI-proteins but a much more general and profound role in signalling via some 'conventional' receptors. Signalling via immunoreceptors [T-cell receptors (TCRs), B-cell receptors (BCRs) and FcRs] is known to be initiated by activation of Src family PTKs, which leads to phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the receptor-associated subunits (CD3 chains, ζ chain family, CD79 chains, FcεR β chain). This results in association of Syk family PTKs to the phosphorylated ITAMs and further phosphorylation and activation of downstream members of signalling cascades such as phospholipase Cγ (PLCγ), phosphoinositide 3-kinase (PI-3-K) and proteins regulating the activity of the small G protein Ras.

There is a basic unexplained point in the above scheme – how are the Src family kinases initially recruited to the immunoreceptor after its interaction with the ligand? It is thought that in the resting state the immunoreceptors are associated with Src family PTKs with low stoichiometry: after crosslinking by their ligands the scarce Src PTKs are sufficient for initial self-phosphorylation-based activation and ITAM

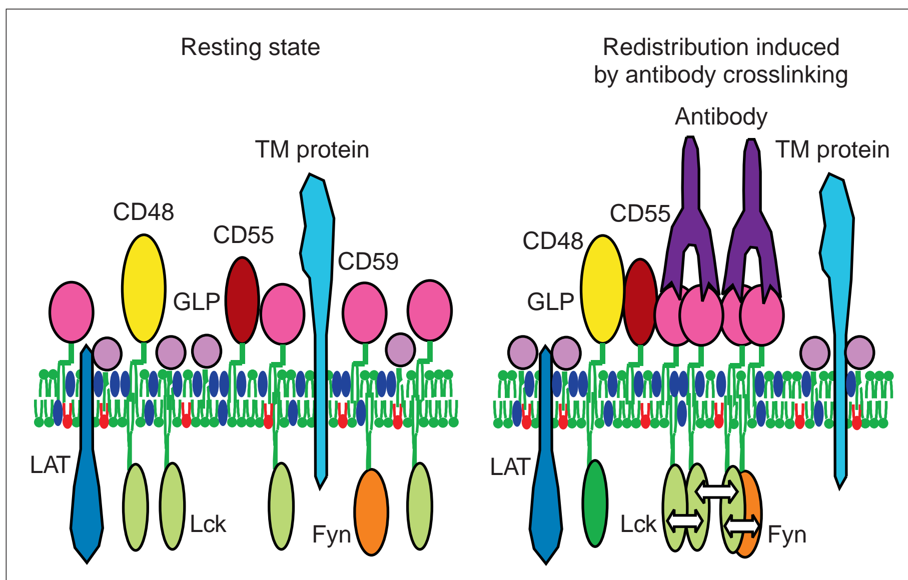

Fig. 3. *Signalling induced by crosslinking of a glycosylphosphatidylinositol (GPI)-anchored protein (here CD59) present in the GPI-microdomains. After antibody-induced redistribution (aggregation) of a GPI-anchored protein residing in a GPI-microdomain, a similar redistribution of the Src family protein tyrosine kinases (PTKs) can be elicited (possibly due to interactions of long acyl chains attached both to the GPI-proteins and Src family PTKs). Aggregated PTKs can start phosphorylating (double-arrows) and thus activating each other and thereby triggering signalling cascades. An alternative possibility is that antibodies aggregate several microdomains and thereby induce PTK redistribution and activation. For abbreviations and key to symbols, see Fig. 2.*

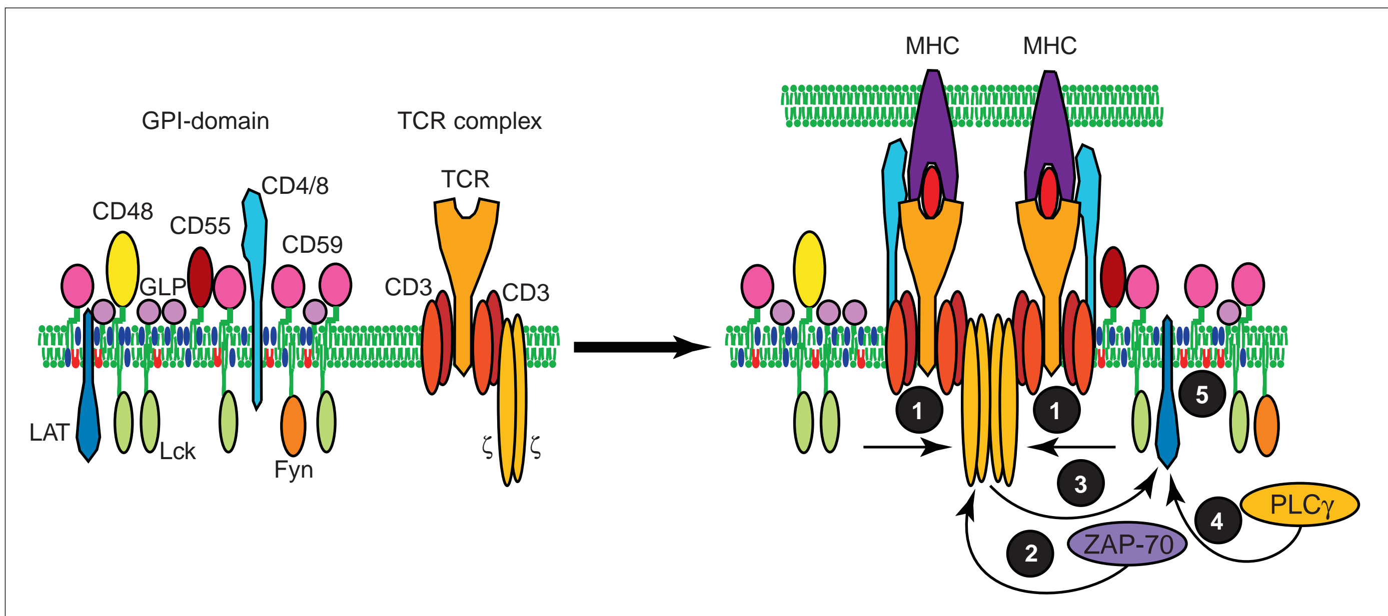

Fig. 4. *Possible role of glycosylphosphatidylinositol (GPI) microdomains in initiation of immunoreceptor (e.g. T-cell receptor) signalling. In the resting state the immunoreceptor complexes are physically separated from the GPI-microdomains. After the immunoreceptor ligation (e.g. upon contact of a T cell with an antigen-presenting cell), the aggregated receptor comes into contact with the protein tyrosine kinase (PTK)-rich GPI-microdomains. The PTKs (possibly after a redistribution-induced activation) phosphorylate the immunoreceptor tyrosine-based activation motif (ITAM) sequences in the immunoreceptor complexes (1). Phosphorylated ITAMs bind and activate Syk family PTKs (ZAP-70) (2), which phosphorylate further substrates including some components of the GPI-microdomains such as the linker protein LAT (3), which binds SH2-containing signalling molecules. One of them is phospholipase Cγ (PLCγ) (4), which becomes subsequently phosphorylation-activated and cleaves PtdIns(4,5)*P_2 *(5). For abbreviations and key to symbols, see Fig. 2.*

phosphorylation, which then attracts further Src family PTKs and Syk family PTKs, mediated via SH2 domains in these kinases. However, recent experimental data suggest an alternative scenario – namely, that immediately after ligation the aggregated immunoreceptors become associated with the GPI-microdomains, and thus become approximated to Src family PTKs that can phosphorylate the ITAMs (Fig. 4). This concept was directly indicated by the results of several recent studies. Field *et al.*[26] observed that, after interaction with the antigen, high affinity FcεRI saturated with specific IgE became rapidly physically associated with detergent-resistant, low-density membrane domains. Another fluorescence microscopic study demonstrated rapid SH2-dependent translocation of FcεRI and signalling molecules (Syk, PLCγ1) to discrete plasma membrane microdomains upon FcεRI-triggered activation of mast cells[27]. Several studies indicate the existence of similar mechanisms in T cells as well. T cells defective in the synthesis of GPI anchors or in expression of specific GPI-anchored proteins exhibit abnormalities in TCR signalling[28,29]. Furthermore, biosynthetic incorporation of polyunsaturated fatty acids in T cells causes displacement of Src family kinases from the GPI-microdomains, which is paralleled by marked inhibition of TCR signalling[30]. Similarly, dispersion of the microdomains by cholesterol-extracting agents impairs early steps of T-cell activation[31]. T-cell activation leads to a striking traffic of TCR and several signal-transducing molecules into the GPI-microdomains[31,32].

The hypothesis for the role of the microdomains in initiation of TCR signalling has gained further support from the identification in them of two other crucial molecules. One of them is phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], a substrate of PLCγ (Ref. 31). The other is a 38 kDa linker protein of activated T cells (LAT), one of the earliest and major tyrosine-phosphorylated proteins following TCR-triggering[33,34]. LAT phosphorylated by activated ZAP-70 binds PLCγ (and probably also other signalling molecules) and thus adjusts it for phosphorylation and activation by the tyrosine kinases present in the microdomains now associated with the TCR complex (Fig. 4). Activated and microdomain-associated PLCγ can now effectively cleave its substrate, PtdIns(4,5)P_2, colocalized in the microdomain. The microdomains were implicated in a recent elegant study as mediators of T-cell costimulation by CD28 engagement[35].

Therefore, we would like to propose that the GPI-microdomains can serve two roles: (1) provide a mechanism by which the GPI-anchored receptors and specific transmembrane proteins constitutively associated with them (such as integrins, as discussed below) transduce signals; and (2) serve as membrane-specialized structures that accumulate Src family kinases and other signalling molecules that can be employed by immunoreceptors and possibly other transmembrane receptors following ligation and aggregation with these 'packages' of signalling molecules (Fig. 4). The major extracellular components of the GPI-microdomains (glycolipids, GPI-anchored proteins) might be involved in the interactions with the immunoreceptors and thus explain the effects on TCR signalling observed in cells deficient in the GPI-anchored proteins[28,29]. Crosslinking of immunoreceptors with antibodies or natural ligands is reported repeatedly to be accompanied by their transition to a detergent-insoluble state; this was traditionally interpreted as association with cytoskeleton[36,37]. However, these results could be due, at least partially, to association of the aggregated immunoreceptors with the detergent-resistant GPI-microdomains[38].

Problems to be solved

Studies on signalling via GPI-anchored proteins have, so far, been an interesting but somewhat esoteric field, as it has not been clear whether the antibody-induced effects mimic the effects of putative natural ligands (and even whether these ligands exist). The recent indications of much more general roles of the GPI-microdomains bring this area into a sharp focus of a broader interest. Several issues still remain to be solved.

Technical considerations

The standard biochemical approach is to solubilize membranes in solutions of mild detergents at low temperature and analyze the resulting complexes. This obviously brings about a danger of certain artefacts – mild detergents at low temperature can either induce associations that do not exist at physiological conditions or can remove certain components (e.g. some transmembrane proteins). It is reassuring that fragments of plasma membrane similar in composition to the detergent-resistant GPI-microdomains can be obtained by a purely mechanical, detergent-free method[39]. Furthermore, a fluorescent-labelled GPI-anchored protein, CD59, can be incorporated into cell membranes and, under physiological conditions, be localized in microscopically observable 'dots', correlating with acquiring signalling capacity[20]. Perhaps most convincing are the results of the recent biophysical and biochemical studies on whole cells[16,17], which clearly support the existence of the GPI-microdomains *in vivo*. Thus, it is reasonable to assume that the GPI-microdomains do exist under physiological conditions[40]; what is less clear is their size, possible heterogeneity and dynamic behaviour in the membrane. In this respect, the technique of 'single particle tracking' appears to be uniquely informative; the results indicate that GPI-anchored proteins and glycolipids are, under physiological conditions, repeatedly and transiently (for 7–9 seconds) confined to membrane regions of increased viscosity (limited diffusion) averaging 300 nm in diameter[41], which might correspond to the GPI-microdomains. Another so far incompletely answered question is identification of all cytoplasmic proteins associated with these membrane structures; it is quite possible that additional novel and important components are yet to be discovered.

GPI-microdomain-associated transmembrane proteins

Another poorly understood point is the role of transmembrane proteins constitutively associated with the GPI-microdomains. In general, most transmembrane proteins appear to be excluded from these structures with the notable exceptions of a fraction of LAT (Refs 33, 34), CD4 and CD8 in T cells[42], integrins in myeloid cells[23], CD44 in various cell types[43,44], CD26 in lymphocytes[44], influenza virus haemagglutinin in epithelial cells[45], and CD36 in platelets[46]. Usually only minor amounts of these proteins reside in the GPI-microdomains. The comparative difference between the molecules found within and outside the microdomains is not yet known, but might be attributable to differences in palmitoylation. Modification by this saturated fatty acid is likely to result in association with the microdomains owing to hydrophobic interactions with the ordered saturated fatty acids of the glycosphingolipids. Integrins can be held in the microdomains owing to their lectin-like interactions with carbohydrate moieties of the GPI-anchored proteins[47,48]. In some cases, the transmembrane proteins could play an important structural role in maintaining proper organization of these membrane specializations (possible linkers between the extracellular and intracellular protein components) or in interactions of the microdomains with other receptors. In this respect the apparently constitutive presence of a fraction of CD4 and CD8 in the microdomains is of potential interest. It is tempting to suggest that interactions of these coreceptors with major histocompatibility complex (MHC) molecules and TCR complex are important in adjoining the microdomains to the ligated TCR.

State of Src family kinases

The functional state of the Src family kinases present in the microdomains and the relationship of the domains to the protein tyrosine phosphatase CD45 remains a controversy. Some results indicate that the microdomain-associated PTKs are in an activated state[39] whilst others suggest that the microdomains harbour hyperphosphorylated, enzymatically inactive Src family kinases that are physically separated from the activating phosphatase activity of CD45 (Ref. 49). It will be important to determine how the activity of Src kinases is regulated in the microdomains and how these islets communicate with CD45.

Concluding remarks

The microdomain hypothesis plausibly explains several so far seemingly unrelated phenomena – signalling capacity of GPI-anchored proteins and glycolipids, striking similarities in the outcome of signalling via GPI-proteins (such as Thy-1) and immunoreceptors, and apparent deficiency of resting immunoreceptors in associated PTKs. GPI-microdomains comprise a remarkable collection of signalling molecules crucial for initiation of TCR signalling [Src family kinases, Ras, PI-3-K, LAT, PtdIns(4,5)P_2]; similar, but as yet less well characterized microdomains appear to play a role in initiation of signalling through high-affinity FcεR and possibly also through other immunoreceptors. The existence of GPI-microdomains might be an example of a more general and so far relatively poorly understood phenomenon – supramolecular organization of plasma membrane into several distinct types of microdomains[50] in which relevant sets of molecules might be accumulated for optimal functional cooperation. For example, existence of microdomains containing TCR and several transmembrane proteins and distinct from the GPI-microdomains has been indicated by a study based on membrane solubilization in mild detergent solutions[51]. It will be interesting to see which other membrane phenomena (signalling through other receptors, endocytosis, exocytosis, vesiculation, interaction with viruses, intercellular adhesion) are influenced by the existence of various types of membrane microdomains. One such potentially important extension of the principle might be the recently suggested involvement

of the microdomains in the phenomenon of immunological co-stimulation and formation of immunological synapses[35]. Finally, it would be desirable to unify the nomenclature of these interesting membrane specializations. Although we have been using the term 'GPI-microdomains' throughout this article, we suggest that for the sake of brevity and for aesthetic and associative reasons the term 'GEMs' is used in future instead of numerous alternative current names.

The work of the Prague laboratory is supported by grant No. 204/99/0367 from the Grant Agency of the Czech Republic; V.H. is supported by an International Research Scholar's Award from the Howard Hughes Medical Institute; the work of the Vienna laboratory is supported by the Austrian Science Fund.

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References

1 Low, M.G. (1989) *FASEB J.* 3, 1600–1608
2 Robinson, P.J. (1991) *Immunol. Today* 12, 35–41
3 Hořejší, V., Cebecauer, M., Černý, J. *et al.* (1998) *Immunol. Lett.* 63, 63–73
4 Swaim, W.D., Minoguchi, K., Oliver, C. *et al.* (1994) *J. Biol. Chem.* 269, 19466–19473
5 Iwabuchi, K., Yamamura, S., Prinetti, A., Handa, K. and Hakomori, S. (1998) *J. Biol. Chem.* 273, 9130–9138
6 Štefanová, I., Hořejší, V., Ansotegui, J., Knapp, W. and Stockinger, H. (1991) *Science* 254, 1016–1018
7 Cinek, T. and Hořejší, V. (1992) *J. Immunol.* 149, 2262–2270
8 Dráberová, L. and Dráber, P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 3611–3615
9 Fra, A.M., Williamson, E., Simons, K. and Parton, R.G. (1994) *J. Biol. Chem.* 269, 30745–30748
10 Parolini, I., Sargiacomo, M., Lisanti, M.P. and Peschle, C. (1996) *Blood* 87, 3783–3794
11 Simons, K. and Ikonen, E. (1997) *Nature* 387, 569–572
12 Rodgers, W., Crise, B. and Rose, J.K. (1994) *Mol. Cell. Biol.* 14, 5384–5391
13 Anderson, R.G.W. (1993) *Curr. Opin. Cell Biol.* 5, 647–652
14 Schnitzer, J.E., McIntosh, D.P., Dvorak, A.M., Liu, J. and Oh, P. (1995) *Science* 269, 1435–1439
15 Iwabuchi, K., Handa, K. and Hakomori, S. (1998) *J. Biol. Chem.* 273, 33766–33773
16 Varma, R. and Mayor, S. (1998) *Nature* 394, 798–801
17 Friedrichson T. and Kurzchalia, T.V. (1998) *Nature* 394, 802–804
18 Schroeder, R., London, E. and Brown, D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 12130–12134
19 Schroeder, R.J., Ahmed, S.N., Zhu, Y., London, E. and Brown, D.A. (1998) *J. Biol. Chem.* 273, 1150–1157
20 Van den Berg, C.W., Cinek, T., Hallet, M.B., Hořejší, V. and Morgan, B.P. (1995) *J. Cell Biol.* 131, 669–677
21 Shenoy-Scaria, A.M., Dietzen, D.J., Kwong, J., Link, D.C. and Lublin, D.M. (1994) *J. Cell Biol.* 126, 353–363
22 Zlatkine, P., Mehul, B. and Magee, A.I. (1997) *J. Cell Sci.* 110, 673–679
23 Bohuslav, J., Hořejší, V., Hansmann, C. *et al.* (1995) *J. Exp. Med.* 181, 1381–1390
24 Hundt, M. and Schmidt, R.E. (1992) *Eur. J. Immunol.* 22, 811–816
25 Štefanová, I., Corcoran, E.M., Horak, L.M. *et al.* (1993) *J. Biol. Chem.* 268, 20725–20728
26 Field, K.A., Holowka, D. and Baird, B. (1997) *J. Biol. Chem.* 272, 4276–4280
27 Stauffer, T.P. and Meyer, T. (1997) *J. Cell Biol.* 139, 1447–1454
28 Hueber, A.O., Bernard, A.M., Battari, C.L.E. *et al.* (1997) *Curr. Biol.* 7, 705–708
29 Romagnoli, P. and Bron, C. (1997) *J. Immunol.* 158, 5757–5764
30 Stulnig, T.M., Berger, M., Sigmund, T. *et al.* (1998) *J. Cell Biol.* 143, 637–644
31 Xavier, R., Brennan, T., Li, Q., McCormack, C. and Seed, B. (1998) *Immunity* 8, 723–732
32 Montixi, C., Langlet, C., Bernard, A-M. *et al.* (1998) *EMBO J.* 17, 5334–5348
33 Brdička, T., Černý, J. and Hořejší, V. (1998) *Biochem. Biophys. Res. Commun.* 248, 356–360
34 Zhang, W., Trible, R.P. and Samelson, L.E. (1998) *Immunity* 9, 239–246
35 Viola, A., Schroeder, S., Sakakibara, Y. and Lanzavecchia, A. (1999) *Science* 283, 680–682
36 Marano, N., Holowka, D. and Baird, B. (1989) *J. Immunol.* 143, 931–938
37 Caplan, S. and Baniyash, M. (1995) *Immunol. Res.* 14, 98–118
38 Marano, N., Crawford, M. and Govindan, B. (1997) *Mol. Immunol.* 34, 967–976
39 Arni, S., Ilangumaran, S., van Echten-Deckert, G. *et al.* (1996) *Biochem. Biophys. Res. Commun.* 225, 801–807
40 Hopper, N.M. (1998) *Curr. Biol.* 8, R114–R116
41 Sheets, E.D., Lee, G.M., Simson, R. and Jacobson, K. (1997) *Biochemistry* 36, 12449–12458
42 Cinek, T., Hilgert, I. and Hořejší, V. (1995) *Immunogenetics* 41, 110–116
43 Perschl, A., Lesley, J., English, N., Hyman, R. and Trowbridge, I.S. (1995) *J. Cell Sci.* 108, 1033–1041
44 Ilangumaran, S., Briol, A. and Hoessli, D.C. (1997) *Biochim. Biophys. Acta* 1328, 227–236
45 Scheiffele, P., Roth, M.G. and Simons, K. (1997) *EMBO J.* 16, 5501–5508
46 Dorahy, D.J., Lincz, L.F., Meldrum, C.J. and Burns, G.F. (1996) *Biochem. J.* 319, 67–72
47 Stockl, J., Majdic, O., Pickl, W.F. *et al.* (1995) *J. Immunol.* 154, 5452–5463
48 Petty, H.R. and Todd, R.F., III (1996) *Immunol. Today* 17, 209–212
49 Rodgers, W. and Rose, J.K. (1996) *J. Cell Biol.* 135, 1515–1523
50 Hwang, J., Gheber, L.A., Margolis, L. and Edidin, M. (1998) *Biophys. J.* 74, 2184–2190
51 Černý, J., Stockinger, H. and Hořejší, V. (1996) *Eur. J. Immunol.* 26, 2335–2343