Minireview

Detergents as tools for the purification and classification of lipid rafts

Luke H. Chamberlain*

Sir Henry Wellcome Laboratory of Cell Biology, Division of Biochemistry and Molecular Biology, Davidson Building, University of Glasgow, Glasgow G12 8QQ, UK

Received 15 December 2003; revised 6 January 2004; accepted 6 January 2004

First published online 22 January 2004

Edited by Lukas Huber

Abstract The relative insolubility of lipid rafts in cold non-ionic detergents is the most widely used method to purify these fascinating membrane domains from intact cells or membranes. Most of what we know about lipid raft function has been derived from experiments utilising detergent insolubility as the basis for raft purification. Recently, a wider range of detergents have been used to purify 'rafts', and rafts have been subclassified based on their differential solubility in different detergents. This minireview critically examines the use of detergents as tools for raft isolation and for the subclassification of rafts.

Key words: Lipid raft; Cholesterol; Sphingolipid; Detergent

1. Introduction

Cell membranes are composed of a diverse array of lipids; how these lipids function to organise membrane proteins and cell pathways is a central question in modern cell biology. There is heterogeneity in the lipid composition of different cellular compartments [1], and lipids can also exhibit selective enrichment at specific regions of the same membrane. For example, the apical plasma membrane of polarised epithelial cells is enriched in (glyco)sphingolipids relative to the basolateral plasma membrane [2]. Simons and van Meer proposed that the distinct lipid compositions of intracellular membranes might provide a mechanism for protein sorting in cells [2]. Specifically, apically destined proteins were suggested to be packaged into sphingolipid-rich vesicles at the *trans*-Golgi network that were then directed to the apical membrane. In support of this idea, apical membrane proteins were shown to co-purify with sphingolipid and cholesterol-rich membranes that were resistant to detergent solubilisation [3]. This detergent resistance was attained shortly after protein synthesis (in the Golgi complex), thus supporting the notion that inclusion of proteins in detergent-insoluble, sphingolipid-rich membrane domains may facilitate sorting to the apical membrane [3]. These findings illuminated the potential formation of lipid-specific domains in cell membranes, and presented an intriguing mechanism for the regulation of protein sorting and other cellular pathways.

*Fax: (44)-141-330 4620.
E-mail address: l.chamberlain@bio.gla.ac.uk (L.H. Chamberlain).

The long acyl chain length, high degree of saturation, and both hydrogen bond acceptor and donor groups of the sphingolipids present in cell membranes promote the tight packing of these lipids. As a result, sphingolipid/cholesterol-rich membranes exist in a 'liquid-ordered' phase, and are less fluid than glycerophospholipid-rich membranes, which are mainly in a 'liquid-disordered' phase. On this basis, the term 'lipid raft' was coined for these cholesterol- and sphingolipid-rich domains [4]. The tight packing and strong interaction of raft lipids have been suggested to explain the resistance of these domains to solubilisation by certain non-ionic detergents [5].

Early studies on lipid rafts relied almost entirely on their purification as detergent-insoluble complexes [3]. As a result, the existence of these domains in vivo was questioned: Did detergent-insoluble complexes purified in vitro reflect the domain distribution of membranes in living cells? Alternatively, were the 'raft' domains purified an artefact of the detergent solubilisation? Subsequent studies on living cells supported the lipid raft hypothesis, demonstrating that 'raft' proteins exhibited cholesterol-dependent clustering at the plasma membrane [6–8]. In addition, studies on model membranes convincingly demonstrated that sphingolipids and cholesterol formed domains in vitro [9–11]. However, it is essential to note that detergents can never be used to purify a transient protein–lipid aggregate in its original form. Likewise, it is doubtful that any other more sophisticated technologies will provide the molecular resolution to precisely extract rafts from their native environment.

Lipid rafts have been implicated in the regulation of numerous cellular events, including signal transduction [12], membrane traffic [13] and viral entry/infection [14]. The large majority of these studies have relied on the detergent insolubility of lipid rafts for their effective purification. The most widely used detergent in these studies is Triton X-100, although more recently a wider spectrum of detergents has been utilised to purify rafts; these studies have lead to the suggestion that insolubility in different detergents reflects the association of proteins with distinct types of 'raft' domain.

2. Purification of Triton X-100-insoluble lipid rafts

Purifying rafts based on their intrinsic detergent insolubility is in theory a simple procedure, and the most commonly used detergent for these studies is Triton X-100. Nevertheless there are still a number of caveats and potential pitfalls. It is essential to note that rafts are only insoluble relative to non-rafts; at high detergent-to-lipid ratios lipid rafts are effectively solu-

0014-5793/04/$30.00
doi:10.1016/S0014-5793(04)00050-X

bilised [15]. Furthermore, raft proteins display different levels of detergent insolubility [16,17]; as a result, detergent titration experiments are essential when assessing the potential interaction of a protein with lipid rafts. A sensible starting point is to find the *lowest* amount of detergent that completely solubilises non-raft proteins (transferrin receptor, Na,K-ATPase) whilst leaving raft proteins insoluble. However, even this approach is not foolproof, as a number of proteins shown to be raft associated by other criteria are completely solubilised by detergent (e.g. the insulin receptor, [18]). Thus, the absence of a protein in detergent-insoluble fractions is not proof of its absence from lipid raft domains in vivo.

Where a protein is found in detergent-insoluble fractions the effects of cholesterol disruption on this insolubility provide a more stringent test of raft association. However, the detergent insolubility of raft proteins and lipids does display differential sensitivity to cholesterol depletion [19]. The purification of rafts with detergents such as Triton X-100 is performed at 4°C; this is essential, as raft proteins are generally solubilised in Triton X-100 at 37°C [3]. The differential solubility of rafts at these two temperatures probably reflects the tighter packaging of membrane lipids as temperature is decreased. However, a number of investigators have questioned whether rafts exist at physiological temperature or whether their formation is a consequence of temperature-induced effects on lipid packing and distribution. In addition, there is evidence that Triton X-100 promotes domain formation in lipid mixtures [20,21]. However, despite these concerns the lipid composition of detergent-insoluble membranes isolated at 4°C may resemble the phase distribution of lipids at 37°C, at least in model membranes [22].

Although Triton X-100 is the best characterised and most widely used detergent for raft purification, there are a number of problems associated with its use. In particular, aggregation of detergent-insoluble raft domains has been demonstrated following Triton X-100 extraction of cells, making estimates of the size and composition or rafts in vivo difficult [16,23]. In addition, a number of proteins shown to be raft associated by other criteria are effectively solubilised by Triton X-100 [12].

3. Raft isolation using detergents other than Triton X-100

Current estimates of the size of lipid raft domains in living cells are in the low nanometre range, and it has been suggested that a basic raft unit may consist of a protein surrounded by a sphingolipid/cholesterol-rich 'lipid shell' [24]. Thus, the number of physically distinct lipid raft domains in living cells is likely to be enormous. Although Triton X-100 is the most widely used detergent for purification of rafts, other detergents have been used for this purpose, including CHAPS, Triton X-114, Lubrol WX, Brij96 and Brij98 [3,16,25,26] (Table 1 compares the properties of some of these detergents used for raft isolation). Can detergent solubilisation of cells be used to purify compositionally distinct lipid raft domains?

Differential solubility of two glycosylphosphatidyinositol-anchored proteins in the same detergent mixture was suggested to reflect their association with distinct lipid raft domains [16]. Thy-1 and prion protein (PrP) were differentially solubilised in a mixture of Brij96 and sodium deoxycholate. Furthermore, the proteins were clustered in separate domains on the neuronal cell surface. Intriguingly, detergent-insoluble raft complexes isolated using Brij96 were suggested not to aggregate following detergent extraction, in contrast to rafts prepared using Triton X-100 [16]. This allowed Thy-1- and PrP-containing rafts to be purified from each other and from the total detergent-insoluble membrane pool [27]; lipid analysis of these detergent-insoluble fractions revealed that PrP rafts had higher levels of cholesterol, hexosylceramide (a glycosphingolipid) and mono-unsaturated phosphatidylcholine and sphingomyelin than Thy-1 rafts. The results from these studies suggest that lipid composition can vary in distinct protein-containing rafts, and, furthermore, that detergent insolubility may be used as a basis to separate distinct types of rafts. However, as a rule, differential insolubility of two proteins in the same detergent does not imply their association with distinct types of raft. For example, the insulin receptor has been shown by a range of techniques to be associated with caveolae, a specialised type of raft that is intrinsically insoluble in Triton X-100; nevertheless, the insulin receptor is effectively solubilised from these domains [18]. Unless the insulin receptor is localised to a specific Triton X-100-soluble subset of caveolae, this suggests that proteins in the same raft domain can display markedly different levels of detergent solubility.

The detailed analysis of Thy-1- and PrP-containing rafts highlights the potential to physically separate and characterise distinct raft domains purified by detergent insolubility. This work also demonstrates that rafts with distinct lipid profiles can be isolated. Is it possible, therefore, that differential solubility of proteins in different detergents could reflect their association with distinct lipid rafts domains? i.e. can a specific detergent selectively purify (by insolubility) a specific raft domain? The pentaspan protein prominin is localised to microvilli at the apical cell surface of Madin Darby canine kidney (MDCK) cells. In contrast to a number of other apically targeted plasma membrane proteins, prominin was soluble in Triton X-100. However, prominin was shown to be partly insoluble in another non-ionic detergent, Lubrol WX; this insolubility was cholesterol dependent [25]. Association with Lubrol-resistant rafts was suggested to facilitate the targeting of prominin specifically to microvilli at the apical cell surface. The authors suggested that insolubility in Lubrol and solubility in Triton X-100 were a consequence of prominins association with a novel lipid raft domain. Subsequent work also implicated Lubrol-resistant rafts in apical targeting in polar-

Table 1
Summary of properties of common detergents used for isolation of detergent-insoluble lipid rafts

Detergent	Solubilisation strength [30]	Raft aggregation [16,26]	Temperature [5,16,25,26]	Cholesterol content [30,33]
Triton X-100	+++	yes	4°C	+++
Brij96	++	no	4°C	+
Brij98	++	no	37°C	+
Lubrol WX	+	N.D.	4°C	+/−

References for the appropriate studies are indicated. N.D. = not determined.

ised hepatic cells [28]. Lubrol rafts were implicated in the direct targeting of proteins to the apical cell surface, whereas Triton-insoluble rafts were shown to function in the indirect (via basolateral cell surface) pathway for apical targeting. As Triton X-100 extraction does not preserve the raft association of a number of proteins [12], the identification of Lubrol as a detergent that can isolate Triton-soluble raft proteins appears to be an important development.

A further recent development in the biochemical isolation of lipid rafts came from a study that used the polyoxyethylene ether Brij98 to solubilise thymocytes [26]. This detergent was chosen because of its mono-unsaturated ether moiety, which was predicted to partition into and preferentially solubilise loosely packed fluid phase lipids. Brij98-resistant rafts were found to be enriched in cholesterol, sphingolipids and palmitoylated proteins, whereas prenylated proteins were effectively solubilised. Thus, Brij98-resistant rafts appear to be compositionally similar to rafts isolated using Triton X-100; however, similar to Lubrol, Brij98 does not solubilise all Triton X-100-soluble proteins [29]. A major advantage that Brij98 has over Triton X-100 is that it isolates raft domains present at 37°C, and is therefore suggested to purify a more 'physiological' pool of rafts [26]. In addition, Brij98 detergent-insoluble membranes were also suggested not to aggregate following detergent extraction, a property that will facilitate their compositional analysis.

4. Novel rafts, the same raft or ineffectual solubilisation?

As Triton X-100 extraction of cells is thought to solubilise a number of raft-associated proteins [12], the use of detergents that isolate Triton X-100-soluble proteins may represent an important development. Furthermore, the use of detergents that do not promote aggregation of lipid rafts is likely to facilitate a more rigorous analysis of protein and lipid compositions of distinct lipid raft domains. However, as with any new tool, the effectiveness of these detergents in isolating specific raft domains requires critical examination.

Despite suggestions to the contrary, at present, differential insolubility of proteins in different detergents is not sufficient to imply their association with distinct lipid rafts. In the absence of any supporting evidence, such differential insolubility may simply reflect a different strength of interaction with the same raft domain (as shown in Fig. 1A). However, if there are data to also show that two proteins are physically separated in intact membranes then it is possible to conclude that the pro-

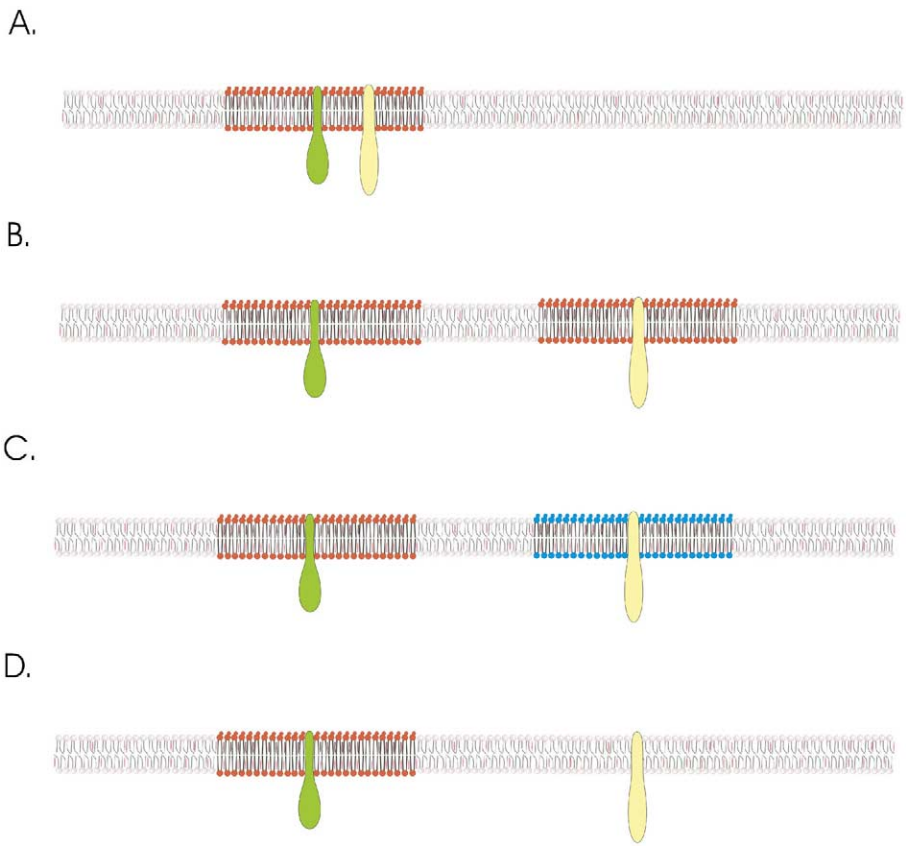

Fig. 1. What does the differential solubility of a protein in different detergents suggest? A: Where there is a lack of morphological evidence that two raft proteins are separated in intact membranes, differential solubility is not sufficient to suggest that the proteins are in distinct rafts. In such a case the proteins may colocalise in the same raft domain but be differentially solubilised from it. B: Differential solubility of raft proteins may be related to the type of interaction with lipid rafts. If there is also morphological evidence that the two proteins are physically separated within intact membranes, then it may be concluded that the proteins are in separate raft domains. However, this does not imply that the proteins are in rafts with different lipid compositions, as the proteins may be physically separated in rafts of the same type. C: Differential solubility of two different proteins may reflect their association with compositionally distinct raft domains (shown in red or blue). However, further evidence is required to support this conclusion [26]. D: If the detergents used do not effectively solubilise membranes, then a protein of interest may not be associated with raft domains at all (shown for yellow protein).

teins are in distinct rafts, but these rafts may have a similar lipid composition (Fig. 1B). To conclude that two proteins are in rafts with distinct lipid compositions (Fig. 1C) requires analysis of individually purified raft domains [27].

Although not preserving all raft-associated proteins, Triton X-100 has the major advantage that solubilisation of the bulk plasma membrane can be assessed by monitoring the solubility of non-raft protein markers such as the transferrin receptor and the Na/K-ATPase. How effective are these 'newer' detergents at solubilising cell membranes? A significant worry is that these 'new' detergents are chosen on the basis that they do not solubilise a protein that *is* soluble in Triton X-100, thus there is a tendency to use weaker detergents. Indeed Triton X-100-resistant rafts are also insoluble in Lubrol WX.

A recent study emphasised the ineffectiveness of Lubrol WX in solubilising plasma membrane proteins [30]; Lubrol-insoluble domains contained >10-fold more biotinylated plasma membrane proteins in MDCK cells than Triton X-100-insoluble domains. Furthermore, although Lubrol WX was initially shown to solubilise the transferrin receptor in MDCK cells [25], more recent studies reported this protein to be insoluble in Lubrol in both MDCK and in multidrug-resistant cell lines [30,31]. The presence of the transferrin receptor in Lubrol-insoluble rafts suggests two main possibilities: (i) This classical 'non-raft' protein is actually raft associated but is solubilised by Triton X-100. However, note that other detergent-independent raft studies have also concluded that the transferrin receptor is a non-raft protein [8,32]. (ii) Lubrol WX is ineffective at solubilising the bulk plasma membrane (Fig. 1D). Brij96- and Brij98-insoluble rafts also contained significantly more plasma membrane proteins than Triton X-100-resistant domains, although these detergents were more effective solubilisers than Lubrol [30]. As Brij98 is a new type of detergent used to purify rafts, its ability to effectively solubilise membranes requires further and more rigorous examination. Although Brij98 solubilises prenylated proteins, it is important to show that classical 'non-raft' *transmembrane* proteins such as the transferrin receptor and the Na/K-ATPase are also solubilised.

The cautionary note concerning the use of Lubrol/Brij98 to purify rafts is further emphasised by recent work analysing the lipid composition of rafts isolated using different detergents [30]. This analysis revealed that Triton X-100-insoluble domains had a marked enrichment of sphingolipids and cholesterol relative to glycerophospholipids, as expected of raft domains. Brij98, on the other hand, isolated domains with only a modest enrichment in sphingolipids/cholesterol. In contrast, Lubrol-resistant rafts had a similar overall lipid composition to total cell membranes. Thus, the lipid profile of Lubrol-resistant rafts suggests that this detergent fails to isolate domains with the characteristics of rafts (i.e. cholesterol/sphingolipid-rich). An independent report also demonstrated that Lubrol rafts were enriched in unsaturated phosphatidylcholine and had a significantly lower cholesterol:cholinephospholipid ratio than Triton-insoluble rafts [33]. If this lipid composition indeed reflects the unique make-up of Lubrol rafts, then it may be necessary to redefine the term 'raft', currently used to describe cholesterol/sphingolipid-rich domains. Similarly, the significantly lower cholesterol/sphingolipid levels in Brij98 rafts suggest that this detergent either isolates a novel class of rafts, or that it does not solubilise the bulk plasma membrane efficiently.

5. Conclusions and perspectives

The aim of this minireview is to highlight the potential problems associated with classifying rafts based on solubility/insolubility in different detergents. These detergents can be useful to identify 'raft' proteins only when there is evidence that the cellular compartment of interest is effectively solubilised (i.e. show that specific integral membrane proteins are soluble). However, the differential behaviour of proteins in distinct detergents, by itself, can not be used to imply association with specific types of raft domain. Even when there is evidence to show that two proteins are spatially separated on the membrane and that they respond differently to agents that disrupt lipid rafts this does not prove their existence in distinct types of lipid rafts (only in distinct rafts). An obvious problem when using Triton X-100 to isolate rafts is the solubilisation by this detergent of significant amounts of raft proteins. Thus detergents such as Lubrol WX may be useful in identifying proteins with a weak affinity for rafts; for example the microvillar localisation of prominin was shown to be cholesterol dependent, suggesting that this protein may be a bona fide raft component, albeit a Triton-soluble one. In addition, isolating rafts with detergents such as Brij98 and Brij96 has advantages, not least that Brij96/98-resistant rafts have been suggested not to aggregate following cell solubilisation [16,26]; aggregation of rafts purified using Triton X-100 prevents rigorous analysis of protein segregation in distinct raft domains. The idea that raft domains represent a minor fraction of cell membranes has been challenged [19], and Triton X-100-insoluble raft proteins may only reflect a subset of proteins that are raft associated in vivo. The challenge now is to develop more robust methods that allow rafts to be isolated in the purest form possible from the bulk plasma membrane. It is likely that further optimisation of detergent-based purifications will permit the development of improved methodology for raft isolation. However, to achieve a comprehensive analysis of the complexities of lateral membrane compartmentalisation undoubtedly requires a unifying molecular picture created from several diverse but complementary approaches.

Acknowledgements: I thank Dr Declan James (University of Glasgow) for preparation of figures, and Christine Salaün (University of Glasgow) and Deborah Brown (SUNY at Stony Brook, NY) for helpful comments. Work in my laboratory is funded by the Wellcome Trust, the Biotechnology and Biological Sciences Research Council, Diabetes UK, the Diabetes Research and Wellness Foundation, and Tenovus.

References

[1] Sprong, H., van der Sluijs, P. and van Meer, G. (2001) Nat. Rev. Mol. Cell Biol. 2, 504–513.
[2] Simons, K. and van Meer, G. (1988) Biochemistry 27, 6197–6202.
[3] Brown, D.A. and Rose, J.K. (1992) Cell 68, 533–544.
[4] Simons, K. (1997) Nature 387, 569–572.
[5] London, E. and Brown, D.A. (2000) Biochim. Biophys. Acta 1508, 182–195.
[6] Friedrichson, T. and Kurzchalia, T.V. (1998) Nature 394, 802–805.
[7] Varma, R. and Mayor, S. (1998) Nature 394, 798–801.
[8] Pralle, A., Keller, P., Florin, E-L., Simons, K. and Horber, J.K.H. (2000) J. Cell Biol. 148, 997–1007.
[9] Schroeder, R., London, E. and Brown, D.A. (1994) Proc. Natl. Acad. Sci. USA 91, 12130–12134.
[10] Ahmed, S.N., Brown, D.A. and London, E. (1997) Biochemistry 36, 10944–10953.
[11] Dietrich, C., Bagatolli, L.A., Volovyk, Z.N., Thompson, N.L.,

Levi, M., Jacobson, K. and Gratton, E. (2001) Biophys. J. 80, 1417–1428.
[12] Simons, K. and Toomre, D. (2000) Nat. Rev. Mol. Cell Biol. 1, 31–41.
[13] Ikonen, E. (2001) Curr. Opin. Cell Biol. 13, 470–477.
[14] Chazal, N. and Gerlier, D. (2003) Microbiol. Mol. Biol. Rev. 67, 226–237.
[15] Chamberlain, L.H. and Gould, G.W. (2002) J. Biol. Chem. 277, 49750–49754.
[16] Madore, N., Smith, K.L., Graham, C.H., Jen, A., Brady, K., Hall, S. and Morris, R. (1999) EMBO J. 18, 6917–6926.
[17] Prior, I.A., Harding, A., Yan, J., Sluimer, J., Parton, R.G. and Hancock, J.F. (2001) Nat. Cell Biol. 3, 368–375.
[18] Gustavsson, J., Parpal, S., Karlsson, M., Ramsing, C., Thorn, H., Borg, M., Lindroth, M., Peterson, K.H., Magnusson, K-E. and Stralfors, P. (1999) FASEB J. 13, 1961–1971.
[19] Hao, M., Mukherjee, S. and Maxfield, F.R. (2001) Proc. Natl. Acad. Sci. USA 98, 13072–13077.
[20] Heerklotz, H. (2002) Biophys. J. 83, 2693–2701.
[21] Heerklotz, H., Szadkowska, H., Anderson, T. and Seelig, J. (2003) J. Mol. Biol. 329, 793–799.
[22] De Almeida, R.F.M., Fedorov, A. and Prieto, M. (2003) Biophys. J. 85, 2406–2416.
[23] Mayor, S. and Maxfield, F.R. (1995) Mol. Biol. Cell 6, 929–944.
[24] Anderson, R.G.W. and Jacobson, K. (2002) Science 296, 1821–1825.
[25] Röper, K., Corbeil, D. and Huttner, W.B. (2000) Nat. Cell Biol. 2, 582–592.
[26] Drevot, P., Langlet, C., Guo, X-J., Bernard, A-M., Colard, O., Chauvin, J-P., Lasserre, R. and He, H.-T. (2002) EMBO J. 21, 1899–1908.
[27] Brügger, B., Graham, C., Leibrecht, I., Mombelli, E., Jen, A., Wieland, F. and Morris, R. (2003) J. Biol. Chem., in press.
[28] Slimane, T.A., Trugnan, G., van Ijzendoorn, S.C.D. and Hoekstra, D. (2003) Mol. Biol. Cell 14, 611–624.
[29] Holm, K., Weclewicz, K., Hewson, R. and Suomalainen, M. (2003) J. Virol. 77, 4805–4817.
[30] Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A. and Simons, K. (2003) Proc. Natl. Acad. Sci. USA 100, 5795–5800.
[31] Hinrichs, J.W.J., Klappe, K., Hummel, I. and Kok, J.W. (2003) J. Biol. Chem., in press.
[32] Janes, P.W., Ley, S.C. and Magee, A.I. (1999) J. Cell Biol. 147, 447–461.
[33] Drobnik, W., Borsukova, H., Böttcher, A., Pfeiffer, A., Liebisch, G., Schütz, G.J., Schindler, H. and Schmitz, G. (2002) Traffic 3, 268–278.