

Lipid rafts and membrane dynamics

Lawrence Rajendran and Kai Simons*

Max-Planck Institute for Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307, Dresden, Germany

*Author for correspondence (e-mail: simons@mpi-cbg.de)

Journal of Cell Science 118, 1099–1102
Published by The Company of Biologists 2005
doi:10.1242/jcs.01681

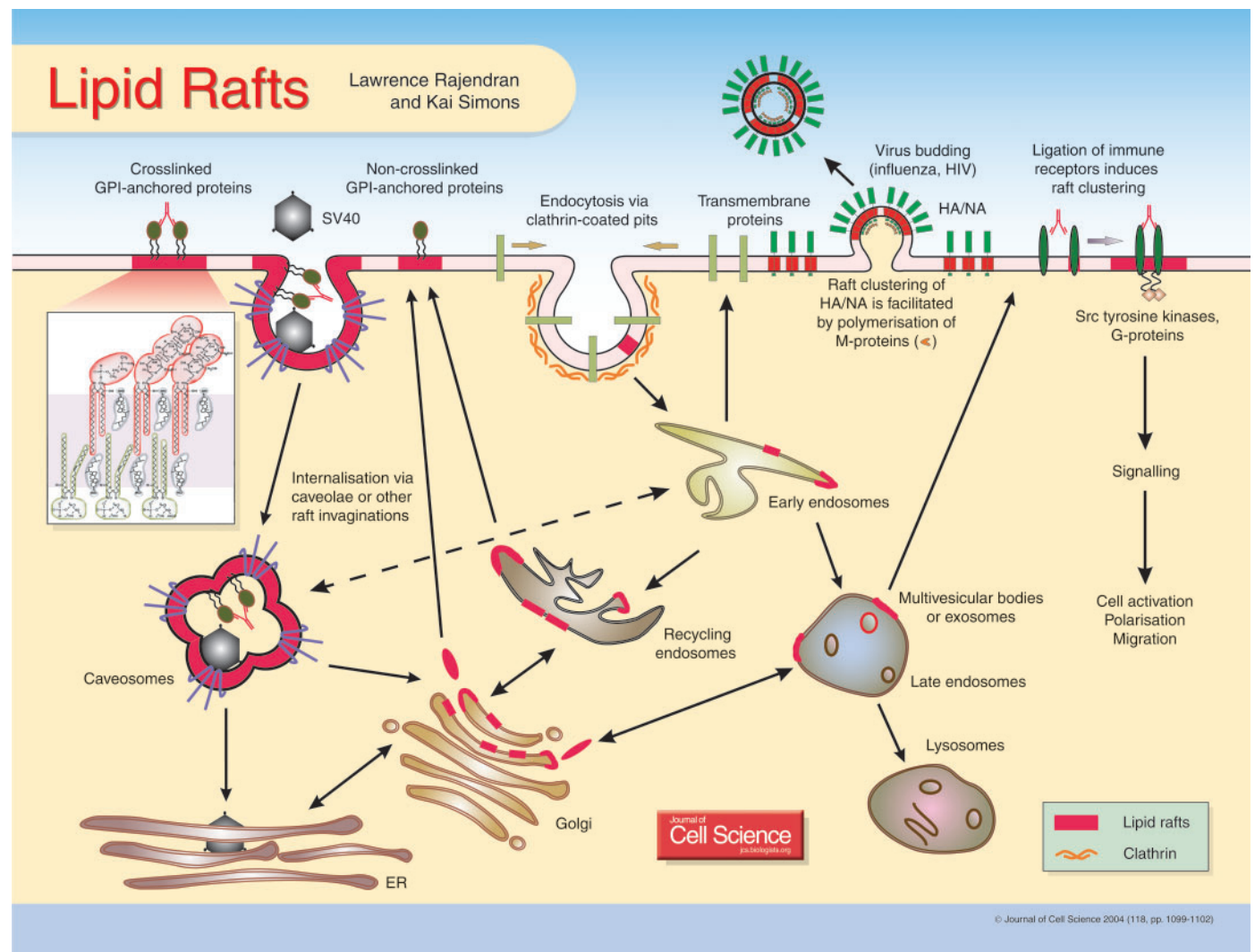
Cell membranes contain a variety of lipid species that differ in their physico-chemical properties. Lipid-lipid immiscibility gives rise to lateral heterogeneities in the membrane plane, a subset of which are termed lipid rafts (Simons and Vaz, 2004). Originally defined biochemically as detergent-

resistant membrane (DRM) fractions, lipid rafts are proposed to be highly dynamic, submicroscopic assemblies that float freely within the liquid disordered bilayer in cell membranes and can coalesce upon clustering of their components. Sphingolipids and cholesterol in the outer exoplasmic leaflet play a crucial role in the assembly of these domains.

Rafts are liquid-ordered domains that are more tightly packed than the surrounding non-raft phase of the bilayer. The tighter packing is due to the saturated hydrocarbon chains in raft sphingolipids and phospholipids compared with the unsaturated fatty acids of phospholipids in the non-raft phase (Simons and Vaz, 2004). Recent studies have suggested that an equivalent domain organisation could be present in

the cytoplasmic leaflet as well. However, the properties of this inner leaflet have not been adequately defined (Parton and Richards, 2003). Theoretical considerations predict that a liquid-ordered packing of the outer leaflet leads to a more ordered packing also of the inner leaflet (Israelachvili, 1973).

Although a large fraction of the cell surface proteins are found in the liquid disordered regions, some proteins preferentially partition into the ordered raft domains. Typical examples include the glycosylphosphatidylinositol (GPI)-anchored proteins, which are attached to the outer leaflet of the membrane via the GPI anchor (Chatterjee and Mayor, 2001); the Src-family tyrosine kinases (e.g. Lck, Fyn and Lyn), which are anchored to the inner leaflet via their dual acylation modification (Simons and



(See poster insert)

Toomre, 2000); palmitoylated and myristoylated proteins such as flotillins (Rajendran et al., 2003); cholesterol-binding proteins such as caveolins (Kurzchalia and Parton, 1999) and hedgehog (Karpen et al., 2001); heterotrimeric G proteins; and phospholipid-binding proteins such as annexins (Babiychuk et al., 2002). One morphologically identifiable raft structure is the caveola (Kurzchalia and Parton, 1999). Caveolae are flask-shaped membrane invaginations found in the plasma membranes of several types of cell enriched in their scaffolding proteins, caveolins. Overexpression of these proteins in cells lacking caveolae, such as lymphocytes and neuronal cells, can induce the formation of caveolae (Fra et al., 1995), and targeted disruption of caveolin 1 in mice leads to the disappearance of morphologically recognisable caveolae. Flotillins are non-caveolar proteins that localise to microdomains and probably function as raft organisers. Annexins have also been reported to organise rafts in a calcium-dependent fashion.

Rafts are dynamic and this means that both proteins and lipids can move in and out of raft domains with different partitioning kinetics. Despite much evidence supporting the existence of raft domains, the size and the functions of these domains are debated (Edidin, 2003). The controversy mainly arises because these domains are too small to be optically resolved. However, recent advances in imaging are now providing insights into their behaviour (Gaus et al., 2003; Parton and Richards, 2003; Pralle et al., 2000). Depending on the time-resolution of the technique used, different properties can be revealed (Kusumi et al., 2004).

The first method to biochemically define lipid rafts was based on the resistance of lipid rafts to extraction by Triton X-100 at 4°C (Brown and Rose, 1992). These DRM fractions are aggregates of raft domains and thus do not represent the native state of lipid rafts in cell membranes (Munro, 2003). One confusion in this field was caused by the equation of lipid rafts to caveolae (Anderson, 1998). Caveolins are clearly a part of DRMs in cells that express these proteins but form a subclass of

rafts (as explained above). A number of new methods are being introduced to study rafts in cells and this field needs better methodology if we are to come to grips with these elusive membrane domains.

At steady state, rafts are too small to engage in raft-associated processes. Whatever their size is, researchers agree that these domains contain only a few proteins. To engage in membrane function, they usually have to cluster together. There is increasing evidence that the outer leaflet domains and the inner leaflet domains are coupled in raft clusters (Gri et al., 2004). Raft clustering can be accomplished from both sides of the plasma membrane. Antibodies, antigens or raft-lipid-binding proteins such as cholera toxin B, cluster rafts on the extracellular side of the membrane whereas raft-clustering proteins such as annexins, flotillins or other scaffolding proteins could serve as clustering agents for the rafts in the cytoplasmic leaflet. Clustered rafts can sequester specific sets of signalling and other proteins and could serve as platforms to execute functions in membrane trafficking, signalling and polarisation (Simons and Toomre, 2000; Harder and Engelhardt, 2004). We review some examples below, starting with membrane trafficking, in which rafts could play an important role as sorting platforms at various stages of the endo- and exo-cytic pathways.

Endocytosis

Caveolae-mediated endocytosis

Recent work has established an endocytic role for caveolae and lipid rafts (Parton and Richards, 2003). Crosslinked GPI-anchored proteins have been shown to translocate to caveolae and subsequently become endocytosed. Moreover, some non-enveloped viruses, such as SV40, use caveolae as portals for entry into the cell. Dynamin, a regulatory GTPase implicated in endocytosis, has been shown to be transiently recruited to virus-loaded caveolae and aid in the internalisation of these structures. Once internalised, the caveolar vesicles seem to deliver the viruses to newly discovered organelles called caveosomes (Pelkmans and Helenius, 2002). These pre-existing

cytoplasmic structures have a neutral pH and are enriched in caveolin 1 but devoid of markers of other endocytic and biosynthetic organelles. From these structures, the viruses are delivered to the ER. Interestingly, there also seems to be crosstalk between early endosomes and the caveosomes. Cholera toxin, folic acid, autocrine motility factor and lactosyl ceramide have also been shown to be internalised via a caveolar endocytic pathway (Pelkmans and Helenius, 2002). Cholesterol depletion of raft components redirects them to other internalisation routes (Shogomori and Futerman, 2001), demonstrating the plasticity of endocytic mechanisms.

Non-caveolar internalisation routes of raft proteins

Certain raft-associated proteins have been shown to be endocytosed by clathrin-mediated endocytosis – the other major mode of internalisation – although this pathway mostly excludes lipid rafts (Nichols, 2003). It is possible that strong endocytic signals trap proteins into clathrin-coated pits and thus enable the proteins to bypass raft-mediated internalisation (Stoddart et al., 2002). GPI-anchored proteins in particular can present complicated scenarios. For example, GPI-anchored CD14 is sorted in a cell-type-specific fashion. While CD14 is sorted to recycling endosomes in CHO cells, it is routed to late endosomes in BHK fibroblasts (Fivaz et al., 2002).

In addition, there is evidence for other raft-mediated routes of internalisation (Lamaze et al., 2001; Sabharanjak et al., 2002). Sabharanjak et al., have described a novel raft-dependent pathway in which native GPI-anchored proteins are internalised to recycling endosomes bypassing the early sorting endosomes but via a newly identified organelle called the GPI-anchored protein enriched early endosomal compartment (GEEC). This compartment has been shown to be devoid of caveolins but accumulates the fluid-phase marker dextran along with the folate receptor. Internalisation to GEECs depends on Cdc42, a Rho GTPase, but neither clathrin nor dynamin is involved. The GPI anchor has been shown to be a GEEC-targeting

signal: a transmembrane equivalent fails to accumulate in the compartment indicating that GEEC-mediated internalisation is a key endocytic pathway for non-crosslinked GPI-anchored proteins.

Interleukin receptor-2 (IL-2R), an essential lymphocyte growth factor has by contrast been shown to be constitutively associated with lipid rafts and uses a non-clathrin-mediated process for internalisation (Lamaze et al., 2001). Its internalisation process depends on dynamin activity but is independent of caveolae. The reason for several distinct raft-mediated endocytic pathways is not yet clear. It is plausible that distinct lipid raft domains, differing not only in their lipid composition but also in the nature of the proteins partitioned into these domains, employ distinct mechanisms.

Sorting in polarised epithelial cells

Polarised epithelial cells direct distinct cargos to their apical and basolateral surfaces, and lipid rafts probably play an important role in polarised membrane trafficking. For most of the GPI-anchored proteins and apical transmembrane proteins, N-glycans or O-glycans attached to the proteins are thought to be the apical sorting signals. N-glycans might bind to putative raft-associated lectins to ensure apical delivery of the protein. Alternatively, certain membrane anchors and transmembrane domains might allow certain proteins to partition into rafts directly, and GPI anchors should generally pack more favourably into liquid ordered domains than disordered regions of the membrane (Schuck and Simons, 2004). Lipid modifications and motifs in the cytoplasmic domains of membrane proteins, such as PDZ-binding motifs, have also been suggested to play roles in apical sorting. The former could also promote raft association and thereby apical sorting. Basolateral delivery, by contrast, depends on targeting signals such as di-leucine motifs or tyrosine motifs that bind to specific cargo receptors. Since basolaterally targeted proteins are also glycosylated, it is assumed that most basolateral-targeting signals possess a higher affinity for their

adaptors than N-glycans do for the putative raft-localised lectins.

Rafts could also play an important role in the formation of transport carriers. Domain-induced budding could provide the driving force for the formation of apical containers. Such a mechanism is proposed to involve outward bending of raft clusters and fission at the domain boundary (Schuck and Simons, 2004).

Role of rafts in virus budding

When viruses bud from host cells, they acquire their membrane from the host cell. Hemagglutinin (HA) and neuraminidase (NA), two spike glycoproteins in influenza A viruses, are raft associated (Scheiffele et al., 1997). Furthermore, lipid analysis of the virus has demonstrated an enrichment of raft lipids, indicating that these viruses might bud from raft domains (Takeda et al., 2003). Another viral protein, the M-protein, is thought to aid the budding of the viruses by binding to the cytoplasmic domain of the spike glycoproteins, which probably induces a conformational change in the M-protein leading to its oligomerisation. This polymerisation not only induces a change in membrane curvature but also leads to clustering of rafts through the spike proteins. Note also that, when M-proteins are expressed alone, they associate with internal membranes and become detergent soluble whereas, when expressed together with the spike glycoproteins, these proteins become detergent resistant (Zhang et al., 2000). This shows that interaction of the M-proteins with the spike glycoproteins and clustering of rafts through polymerisation of M-proteins represents a key event in the budding of the viruses.

HIV employs a different mechanism to exit the host cell, using the endosomal sorting complexes required for transport (ESCRT) machinery normally responsible for the formation of the internal vesicles in multivesicular bodies (MVBs) for assembly of its envelope. The site of assembly could be either the plasma membrane or MVBs (von Schwedler et al., 2003). Nevertheless, it has been shown that raft lipids play a role in this process (Aloia et al., 1988). Most importantly, the HIV envelope is

enriched in raft lipids, and both entry and exit of the virus is dependent on functional rafts.

Rafts in immune receptor signalling

Much of the early evidence for a functional role of lipid rafts came from studies of hematopoietic cells. Multi-chain immune receptors include the high-affinity IgE receptor (FcεRI), the T-cell receptor (TCR) and the B cell receptor (BCR). These receptors translocate to lipid rafts upon crosslinking (Sedwick and Altman, 2002). Aggregation of FcεRI by antigen, for example, causes its translocation to DRM patches and activation of the associated Src-family kinase Lyn, which initiates a signalling cascade that culminates in degranulation (Field et al., 1995). Similarly, upon engagement of the TCR, the Src-like tyrosine kinases Lck and Fyn become activated. This leads to their recruitment to the receptor complex, activation of the tyrosine kinase Zap-70, and phosphorylation of adaptor molecules such as LAT and SLP-76. Several GPI-anchored proteins and signalling adaptors then amplify TCR signalling (Horejsi, 2003). Lck, LAT and certain other proteins constitutively associate with lipid rafts at least in part because of their fatty acid modifications. This facilitates propagation of signalling.

When T cells recognise the antigen presented on the surface of antigen-presenting cells (APCs), polarisation of lipid rafts and raft-associated proteins occurs through their association with the immunological synapse, where the T cell contacts the APC (Burack et al., 2002). In addition, T-lymphoblasts exposed to migratory signals develop polarised domains at the actin-rich leading edge and at the trailing edge. Recent studies show that one ganglioside, GM1, localises to the uropods whereas another, GM3, segregates to the leading edge. Two different raft clusters, GM1-rafts and GM3-rafts, containing different subsets of raft-associated proteins therefore become dynamically segregated during cell polarisation (Gomez-Mouton et al., 2004). Lipids rafts thus appear to play a crucial role at the interface between signalling,

membrane trafficking and cell polarisation.

Winchil Vaz and the members of the Simons lab are gratefully acknowledged for the critical comments on the manuscript. Special thanks to Jonas Ries for the help with graphics. Much of the work is supported by the grant (SFB-TR13-04) by the DGF and by the EU grant HPRN-CT-2002-00259.

References

- Aloia, R. C., Jensen, F. C., Curtain, C. C., Mobley, P. W. and Gordon, L. M. (1988). Lipid composition and fluidity of the human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **85**, 900-904.
- Anderson, R. G. (1998). The caveolae membrane system. *Annu. Rev. Biochem.* **67**, 199-225.
- Babiychuk, E. B., Monastyrskaya, K., Burkhard, F. C., Wray, S. and Draeger, A. (2002). Modulating signaling events in smooth muscle: cleavage of annexin 2 abolishes its binding to lipid rafts. *FASEB J.* **16**, 1177-1184.
- Brown, D. A. and Rose, J. K. (1992). Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* **68**, 533-544.
- Burack, W. R., Lee, K. H., Holdorf, A. D., Dustin, M. L. and Shaw, A. S. (2002). Cutting edge: quantitative imaging of raft accumulation in the immunological synapse. *J. Immunol.* **169**, 2837-2841.
- Chatterjee, S. and Mayor, S. (2001). The GPI-anchor and protein sorting. *Cell. Mol. Life Sci.* **58**, 1969-1987.
- Edidin, M. (2003). The state of lipid rafts: from model membranes to cells. *Annu. Rev. Biophys. Biomol. Struct.* **32**, 257-283.
- Field, K. A., Holowka, D. and Baird, B. (1995). Fc epsilon RI-mediated recruitment of p53/56lyn to detergent-resistant membrane domains accompanies cellular signaling. *Proc. Natl. Acad. Sci. USA* **92**, 9201-9205.
- Fivaz, M., Vilbois, F., Thurnheer, S., Pasquali, C., Abrami, L., Bickel, P. E., Parton, R. G. and van der Goot, F. G. (2002). Differential sorting and fate of endocytosed GPI-anchored proteins. *EMBO J.* **21**, 3989-4000.
- Fra, A. M., Williamson, E., Simons, K. and Parton, R. G. (1995). De novo formation of caveolae in lymphocytes by expression of VIP21-caveolin. *Proc. Natl. Acad. Sci. USA* **92**, 8655-8659.
- Gaus, K., Gratton, E., Kable, E. P., Jones, A. S., Gelissen, I., Kritharides, L. and Jessup, W. (2003). Visualizing lipid structure and raft domains in living cells with two-photon microscopy. *Proc. Natl. Acad. Sci. USA* **100**, 15554-15559.
- Gomez-Mouton, C., Lacalle, R. A., Mira, E., Jimenez-Baranda, S., Barber, D. F., Carrera, A. C., Martinez, A. C. and Manes, S. (2004). Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. *J. Cell Biol.* **164**, 759-768.
- Gri, G., Molon, B., Manes, S., Pozzan, T. and Viola, A. (2004). The inner side of T cell lipid rafts. *Immunol. Lett.* **94**, 247-252.
- Harder, T. and Engelhardt, K. R. (2004). Membrane domains in lymphocytes – from lipid rafts to protein scaffolds. *Traffic* **5**, 265-275.
- Horejsi, V. (2003). The roles of membrane microdomains (rafts) in T cell activation. *Immunol. Rev.* **191**, 148-164.
- Israelachvili, J. N. (1973). Theoretical considerations on the asymmetric distribution of charged phospholipid molecules on the inner and outer layers of curved bilayer membranes. *Biochim. Biophys. Acta* **323**, 659-663.
- Karpen, H. E., Bukowski, J. T., Hughes, T., Gratton, J. P., Sessa, W. C. and Gailani, M. R. (2001). The sonic hedgehog receptor patched associates with caveolin-1 in cholesterol-rich microdomains of the plasma membrane. *J. Biol. Chem.* **276**, 19503-19511.
- Kurzchalia, T. V. and Parton, R. G. (1999). Membrane microdomains and caveolae. *Curr. Opin. Cell Biol.* **11**, 424-431.
- Kusumi, A., Koyama-Honda, I. and Suzuki, K. (2004). Molecular dynamics and interactions for creation of stimulation-induced stabilized rafts from small unstable steady-state rafts. *Traffic* **5**, 213-230.
- Lamaze, C., Dujeancourt, A., Baba, T., Lo, C. G., Benmerah, A. and Dautry-Varsat, A. (2001). Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. *Mol. Cell* **7**, 661-671.
- Munro, S. (2003). Lipid rafts: elusive or illusive? *Cell* **115**, 377-388.
- Nichols, B. J. (2003). GM1-containing lipid rafts are depleted within clathrin-coated pits. *Curr. Biol.* **13**, 686-690.
- Parton, R. G. and Richards, A. A. (2003). Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. *Traffic* **4**, 724-738.
- Pelkmans, L. and Helenius, A. (2002). Endocytosis via caveolae. *Traffic* **3**, 311-320.
- Pralle, A., Keller, P., Florin, E. L., Simons, K. and Horber, J. K. (2000). Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. *J. Cell Biol.* **148**, 997-1008.
- Rajendran, L., Masilamani, M., Solomon, S., Tikkanen, R., Stuermer, C. A., Plattner, H. and Ilges, H. (2003). Asymmetric localization of flotillins/reggies in preassembled platforms confers inherent polarity to hematopoietic cells. *Proc. Natl. Acad. Sci. USA* **100**, 8241-8246.
- Sabharanjak, S., Sharma, P., Parton, R. G. and Mayor, S. (2002). GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. *Dev. Cell* **2**, 411-423.
- Scheiffele, P., Roth, M. G. and Simons, K. (1997). Interaction of influenza virus haemagglutinin with sphingolipid-cholesterol membrane domains via its transmembrane domain. *EMBO J.* **16**, 5501-5508.
- Schuck, S. and Simons, K. (2004). Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* **117**, 5955-5964.
- Sedwick, C. E. and Altman, A. (2002). Ordered just so: lipid rafts and lymphocyte function. *Sci. STKE* **2002**, RE2.
- Shogomori, H. and Futerman, A. H. (2001). Cholesterol depletion by methyl-beta-cyclodextrin blocks cholera toxin transport from endosomes to the Golgi apparatus in hippocampal neurons. *J. Neurochem.* **78**, 991-999.
- Simons, K. and Toomre, D. (2000). Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1**, 31-39.
- Simons, K. and Vaz, W. L. (2004). Model systems, lipid rafts, and cell membranes. *Annu. Rev. Biophys. Biomol. Struct.* **33**, 269-295.
- Stoddart, A., Dykstra, M. L., Brown, B. K., Song, W., Pierce, S. K. and Brodsky, F. M. (2002). Lipid rafts unite signaling cascades with clathrin to regulate BCR internalization. *Immunity* **17**, 451-462.
- Takeda, M., Leser, G. P., Russell, C. J. and Lamb, R. A. (2003). Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. *Proc. Natl. Acad. Sci. USA* **100**, 14610-14617.
- von Schwedler, U. K., Stuchell, M., Muller, B., Ward, D. M., Chung, H. Y., Morita, E., Wang, H. E., Davis, T., He, G. P., Cimbara, D. M. et al. (2003). The protein network of HIV budding. *Cell* **114**, 701-713.
- Zhang, J., Pekosz, A. and Lamb, R. A. (2000). Influenza virus assembly and lipid raft microdomains: a role for the cytoplasmic tails of the spike glycoproteins. *J. Virol.* **74**, 4634-4644.

Cell Science at a Glance on the Web
Electronic copies of the poster insert are available in the online version of this article at jcs.biologists.org. The JPEG images can be downloaded for printing or used as slides.