From rafts to crafts: membrane asymmetry in moving cells

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Many important biological events, including the leukocyte-mediated immune response, wound repair, axon guidance and developmental patterning, involve persistent cell movement towards a directional signal, a process termed chemotaxis. Establishment of functional and spatial cell polarity is an absolute requirement for this response. We propose that redistribution of specific membrane microdomains, termed rafts, during cell migration is a pivotal step in achieving polarity. On the one hand, partitioning of molecules into rafts might help to localize proteins at the front or the rear of moving cells, and on the other hand, rafts might function as platforms for local activation and coordination of the signaling pathways involved in cell migration.

Chemotaxis is the process by which cells detect the direction and intensity of, and move toward, an extracellular chemoattractant gradient. To achieve directed movement, a cell must acquire and maintain spatial and functional asymmetry, a process termed polarization [1]. Polarity refers to the ability of a migrating cell to change its morphology in response to chemoattractants and to maintain a stable asymmetric shape with two opposite cell edges: the leading edge, which protrudes at the cell front and the rear edge (termed uropod in leukocytes), which retracts [2]. In mammals, cell polarity is an absolute requirement for cell movement; concurrently, migration reinforces polarization [3].

The basic molecular mechanisms involved in polarization of moving cells resemble those of other cell types, such as neurons or epithelial cells. Membrane microdomains, known as lipid rafts, are a conserved component of this polarization process from yeast to mammalian cells [4–8]. Lipid rafts are thought to assemble through tight packing of the long, highly saturated acyl chains of sphingolipids with sterols [Box 1], forming platforms for polarized protein delivery and membrane compartmentalization. Diverse proteins [e.g. glycosylphosphatidylinositol (GPI)-anchored and double-acylated proteins] associate specifically with lipid rafts and are thus sorted or retained in a polarized fashion. Based on their role in cell polarization, lipid rafts have been implicated in the migration of leukocytes, carcinomas, epithelial cells and nerve growth cones [9–14].

Whereas raft involvement in immune synapse formation is well established [15], the role of these microdomains in cell chemotaxis is poorly understood. Green fluorescent protein (GFP) technology now permits direct examination of raft dynamics in chemotaxing leukocytes, showing that the basic raft units, too small to be seen by light microscopy, become larger rafts that transport specific membrane receptors to concrete sites in migrating cells (C. Gómez-Moutón et al., unpublished). Here, we review the way in which raft clustering helps organize signaling in chemoattractant-stimulated cells, leading to polarity and chemotaxis.

Chemoattractants: spatial cues
Cell polarity is initiated by spatial signals from the extracellular milieu; in the case of chemotaxis, these cues are provided by chemoattractants. Chemoattractants are generally small, water-soluble molecules that function as ligands for cell-surface receptors, transmitting the signal from the environment to the cell interior. Most known chemoattractants for leukocytes, yeast and Dictyostelium bind to seven-transmembrane receptors coupled to heterotrimeric G proteins (G protein-coupled receptors; GPCRs). Nonetheless, other cell types polarize in response to non-GPCR agonists, such as growth factors.

Several reports have suggested that GPCR signaling is fundamental in mediating cell polarity for distinct receptor inputs. The insulin-like growth factor-I (IGF-I) is a potent chemoattractant for breast carcinomas, such as the MCF-7 cell line, through its interaction with the tyrosine kinase receptor IGF-1R. Strikingly, IGF-I-induced chemotaxis is dependent on GPCR signalling [16]. IGF-I stimulation of MCF-7 cells results in increased expression of the chemokine CCL5 (RANTES), which activates its receptor, CCR5 (Fig. 1). Interference with this receptor crosstalk, through inhibition of GPCR signaling, dominant-negative CCR5 mutants or anti-CCL5 neutralizing antibodies, specifically prevents IGF-I-induced polarization and chemotaxis. Basic fibroblast growth factor-induced chemotaxis can be blocked with neutralizing antibodies to the chemokine CCL2 [monocyte chemotactic protein-1 (MCP-1)] and platelet-derived growth factor-induced chemotaxis requires sphingosine phosphate interaction with its receptor, endothelial differentiation gene-1 (EDG-1) [16,17]. These data suggest that GPCR activity is a general requirement for cell polarity and chemotaxis in response to growth factors. Dissection of
GPCR-induced signaling could therefore provide the basis for understanding cell polarization in response to a wide variety of external signals, including GPCR and tyrosine kinase agonists.

**Actin cytoskeleton: linking force for cell polarity and movement**

Chemotaxis requires a concerted dialogue between the cell-polarization and motility machineries [3]. At the single-cell level, migration involves coordinated, dynamic interplay between attachment at the leading edge and detachment at the uropod, combined with traction forces exerted by myosin-based contraction, which push the cell body forward. Both polarity and migration depend to a large extent on the dynamic polymerization of filamentous actin (F-actin) at the leading edge [18]. Immediately after chemotactic stimulation, motile cells extend their leading edges by assembling a branched network of F-actin. Consequently, actin and actin-binding proteins, including talin, α-actinin, vinculin, coronin and the actin-activated protein kinase substrate (IRSp53) overcomes this inhibition [25].

Nonetheless, a major property of rafts is that they can coalesce with other raft units, forming larger platforms. Initial characterization of raft components took advantage of their insolubility in certain non-ionic detergents, enabling them to be separated from glycerolipid-rich membranes. Using this biochemical approach, rafts were shown to selectively include some proteins and exclude others. This property, together with raft unit mobility and coalescence in the membrane plane, can regulate (or increase efficiency) of the interaction among membrane-associated proteins. Because cholesterol is essential for the maintenance of the two membrane phases, extraction of cholesterol from the membrane results in the dispersion of lipid rafts. Cyclodextrins are often used to lower cholesterol levels acutely or, when complexed to cholesterol, to introduce excess cholesterol into cell membranes. Cyclodextrin treatment periods must be limited because when applied to cells for several hours, the drug affects both the plasma membrane and the intracellular organelles connected to it.

![Fig. 1. Growth-factor-induced polarization requires G protein-coupled receptor (GPCR) activation. Several reports suggest that growth factors transactivate GPCR through an extracellular triggering loop involving a GPCR agonist. Here we show the insulin-like growth factor-I (IGF-I)–CCL5 loop described for MCF-7 breast carcinoma cells [16]; IGF-I stimulation (primary input) promotes upregulation of extracellular CCL5 levels (secondary input), activating the chemokine receptor (CCR5) in an autocrine and/or paracrine manner.](http://treimm.trends.com)
Rho GTPases also influence the disassembly of actin filaments. Rac stimulates p21-activated kinases (PAKs), which in turn activate LIM kinase (LIMK) [26]. Activated LIMK then phosphorylates and inactivates coflin, inhibiting actin depolymerization. PAK is also implicated in regulating the myosin II filament contraction required to detach the cell posterior [27], suggesting that PAK is important in coordinating axial polarity in response to chemoattractants.

It is of note that chemotaxing mammalian cells require integrin interaction with the extracellular matrix to establish full polarity [21]. Integrin engagement is also an absolute requirement for epithelial-cell polarity [5], again indicating conservation of the polarization mechanisms among distinct cell types. Integrin engagement controls Rac recruitment to the membrane [28], which could explain the requirement for integrin activation. Moreover, a series of elegant experiments showed that integrins not only regulate membrane recruitment but also restrict Rac activation by displacing the Rho GDP dissociation inhibitor (Rho-GDI), which blocks effector binding [29]. Although chemoattractants could thus activate Rac globally in the cell, integrins would determine the local areas where Rac binds to effectors; this would establish where cells extend lamellipodia, facilitating efficient migration.

Asymmetric distribution of lipid rafts during cell migration

A consequence of cell polarity is the asymmetric localization of membrane receptors, membrane-anchored proteases and signaling molecules between the leading edge and the uropod [2]. Although there are differences among cell types, the leading edge usually contains the machinery that induces localized actin polymerization and that senses the environment. With regard to the latter, there is controversy as to whether or not chemosensory receptors redistribute to the advancing front of migrating cells. Whereas the use of GFP-tagged receptors suggests that chemosensory receptors remain homogeneously distributed on the cell surface [30,31], other groups report that chemoattractant receptors for formyl-methionyl-leucyl-phenylalanine (fMLP), chemokines and epidermal growth factor (EGF) redistribute to the leading edge in polarized cells [9,13,32–35]. The asymmetric distribution of chemo-sensory receptors concurs with the enrichment in G protein β-subunits at the leading edge and their depletion at the uropod [36]. Chemoattractant binding is also increased at the leading-edge compared to the rear edge or sides of polarized cells [37]. Accumulation of these receptors might also explain the greater sensitivity to chemoattractants at the front of polarized moving cells; when the direction of the chemoattractant gradient is changed, polarized cells turn towards the new stimulus source.

Independently of chemoattractant receptor distribution, the preferential generation of the phosphatidylinositol 3 kinase (PI3K) products (PtdIns(3,4,5)P3 and PtdIns(3,4)P2) at the leading edge of polarized and non-polarized cells is reported as an event downstream of G-protein activation [36–41]. Accumulation of these lipids enables localized membrane recruitment of proteins with specific pleckstrin homology (PH) domains, thought to be important in cell sensing of the chemoattractant source. Recent studies in Dictyostelium suggest that chemoattractants induce membrane recruitment of the 3’ lipid phosphatase and tensin homolog (PTEN) at the sides and back of moving cells, helping to restrict PI3K activity to the front [42,43]. Whether PTEN has a role in directional sensing in mammalian cells is controversial, however. Studies using PTEN-null cells suggest that PTEN activity is important in establishing basal PtdIns(3,4,5)P3 and PtdIns(3,4)P2 levels but that it has no role in determining the levels of these lipids following receptor activation [44]. Further, visualization of a GFP-tagged PTEN in moving leukocytes did not show asymmetric distribution (C. Gómez-Moutón et al., unpublished).

Whether or not PTEN distributes to the cell posterior, several receptors and cytoskeletal components are reported to concentrate at the rear of polarized cells [2]. It is worth noting that, whereas the rear edge of fibroblasts seems to be a passive tail, the lymphocyte uropod is a specialized pseudopod-like projection with important motility and adhesion functions. Consequently, several intercellular adhesion molecules (ICAMs) concentrate at the uropod, including ICAM-1, -2 and -3, CD43 and CD44, as well as the actin-binding proteins of the ezrin-radixin-moesin (ERM) family.

Asymmetric distribution of lipid rafts during cell migration

Another asymmetric feature observed at the plasma membrane during polarization involves lipid rafts. Asymmetric distribution of raft domains after chemoattractant stimulation is described in several mammalian-cell types [9–13], as well as in pheromone-stimulated yeast [8]. There is nonetheless controversy as to where and how raft domains localize and function in migrating cells. Leading edge raft accumulation is described in chemoattractant-stimulated cancer cells [9] and in fibroblasts exposed to electrical fields that induce directional movement [13]; conversely, exclusive uropod raft accumulation is reported in fixed T lymphocytes [11] and neutrophils [12]. Two raft subtypes, distinguished by their ganglioside composition, segregate to each cell pole, with leading-edge rafts (L-rafts) enriched in the monosialiganglioside GM3 and uropod rafts (U-rafts) enriched in GM1 [10]. Notably, two distinct raft types are also implicated in pheromone-induced yeast polarization [8].

Analysis of the dynamic redistribution of raft domains in leukocytes engaged in chemotaxis confirmed segregation of distinct raft subtypes during cell migration. Real-time confocal videomicroscopy studies using GFP–GPI as a raft marker indicate that raft domains redistribute to, and persist at, the leading edge and uropod in directionally-stimulated lymphocytes, promyelocytic and neutrophil-like cells (C. Gómez-Moutón et al., unpublished). Raft segregation takes place shortly after chemoattractant stimulation, persists during chemotaxis and depends on actin cytoskeleton integrity. Redistribution of the GFP–GPI marker indicates that proteins with no functional significance in cell polarization or migration distribute asymmetrically in migrating lymphocytes as a function of their raft association. This explains why
polarization of chemosensory receptors, which are raft-associated (see later), appears to be independent of the chemoattractant stimulus and correlates only with the acquisition of a motile phenotype [35].

**Raft segregation as a mechanism for membrane-receptor redistribution in migrating cells**

Evidence indicates that membrane receptor association with distinct raft domains dictates their redistribution to the appropriate location in polarized T cells. Indeed, chemosensory receptors of the chemokine family, such as CXCR4 or CCR5 [10,45–48], the tyrosine kinase EGF receptor [13], membrane-anchored proteases [49,50], CD44 [51] or ICAM-3 [11], among other membrane proteins, are reported to partition in rafts and to be redistributed in migrating cells. Modification of proteins to impede association with rafts inhibits their asymmetrical redistribution in migrating cells [9,10], further reinforcing the role of rafts in specific protein localization during cell polarization. Taken together, these data suggest a model in which raft-domain redistribution causes the polarization of both transmembrane and lipid-linked ordered domain-associated proteins. Known L- and U-raft markers in distinct cell types are outlined in Figure 2.

One important point is to identify the molecular signals that trigger preferential protein association with a specific raft subtype and, consequently, that establish the specific cell location of the protein during migration. Some membrane receptors interact directly with lipid components, determining their partitioning into specific raft subtypes. For instance, the EGF receptor interacts directly with GM3 [52], favoring the association of this receptor with L-rafts and its polarization to the leading-cell edge [13]. Nevertheless, raft redistribution and receptor location might be an actin-driven process. For example, CD44 or ICAMs can form a bridge between ordered domains and the actin cytoskeleton by interacting with raft-associated actin-binding proteins [51]. Indeed, ERM proteins, which link F-actin to the cytoplasmic tails of these adhesion molecules, partition in GM1-enriched rafts and redistribute to the leading edge in fibroblasts but to the uropod in lymphocytes [53]. Some proteins might associate preferentially with rafts of a specific composition; in this way, transmembrane-protein movement could segregate different raft components in opposite directions.

**Raft domains as organizational centers for signaling in chemotaxing cells**

Current evidence suggests that rafts are platforms in which interactions take place between activated receptors and signal-transduction partners in an efficient manner [54]. In migrating cells, rafts might not only increase signaling efficiency but might also restrict and/or organize signaling to specific cell areas. Indeed, the initial partners in chemoattractant-mediated signaling, such as chemosensory receptors and heterotrimeric G proteins, partition in rafts [45,55,56]. More importantly, chemokine-receptor signaling requires association with cholesterol-enriched raft domains [47,48]; in these studies, cholesterol sequestration resulted in a conformational change in the receptors that rendered them unable to bind the ligand. Because chemokine receptors show a preferential affinity for GM3-enriched L-raft domains [10,46], L-raft accumulation at the leading edge could thus deliver ‘active’ receptors, permitting restricted signaling at this cell site.
Membrane rafts could also be the platforms to which certain cytosolic signaling partners are recruited. As mentioned, several reports suggest that PtdIns(3,4,5)P$_3$ and PtdIns(3,4)P$_2$ generation at the leading edge of migrating cells is an early event required for gradient sensing. Recent evidence suggests that raft-domain segregation spatially controls the PI3K signaling pathway during chemotaxis of mammalian cells [15]. GFP-tagged p110$\gamma$ (the PI3K catalytic subunit) and protein kinase B PH domain (PKB-PH) co-localize with raft markers at the leading edge, suggesting that L-rafts are the preferred platforms for PI3K recruitment and activation in moving cells. Concurring with a raft role in PI3K signaling, cholesterol depletion impedes raft redistribution and asymmetric PKB-PH membrane recruitment in cholesterol-depleted cells. Cholesterol-depleted cells emit short-lived pseudopods that are randomly directed independent of the attractant source, suggesting that these cells sense the attractant but cannot interpret the gradient correctly and cannot polarize (C. Gómez-Moutón et al., unpublished).

Rafts might also be involved in the spatial control of the actin network. This has been exemplified in neurons, in which specific raft dispersion at the leading lamella of growth cones impairs axon migration [14]. Nonetheless, the precise mechanisms underlying this control have not been fully defined. Several scenarios could be envisioned as to how rafts control actin assembly. One involves localized PtdIns(4,5)P$_2$ generation in rafts, dependent on membrane cholesterol [23]. Accumulation of PtdIns(4,5)P$_2$ in rafts would also work in concert with raft recruitment of adaptor proteins, such as Nck or Grb2, which link actin polymerization to tyrosine kinase pathways [54]. Grb2 and Nck bind the proline-rich domain of N-WASP and cooperate with PtdIns(4,5)P$_2$ to overcome N-WASP autoinhibition.

Another possibility involves direct regulation of Rho-GTPase recruitment and activity. Rho-GTPases are recruited to the plasma membrane by C-terminal acylation. Although these modifications involve isoprenyl-based lipid anchors, which would exclude them from rafts, several reports implicate rafts in the modulation of Rho-GTPase activity. Indeed, targeting of specific signaling molecules to rafts or crosslinking of raft-associated components promotes Rho activation [57]. Cholesterol depletion impedes prolonged Rac activation in neutrophils, preventing polarization [58]. It is, therefore, possible that rafts regulate Rho-GTPase activity indirectly. Rho-GDI binds directly to the raft-associated CD44 protein [59] and Rho-GDI functions as a switch that affects Rac activity locally [29].

Concluding remarks
There is increasing evidence that compartmentalization of the plasma membrane into distinct microdomains is pivotal in establishing and maintaining cell polarity. During cell polarization, specific raft-microdomain types coalesce to create asymmetries at the plasma membrane. Raft domains are below light microscopy resolution in ‘resting’ cells; when cells are stimulated to polarize, however, rafts assemble into large-scale domains that can be visualized at specific cell locations by light microscopy. The use of drugs that sequester membrane cholesterol or prevent domain segregation indicates that this transition is required to achieve polarity in migrating cells [9,10,58]. Segregation of specialized raft domains could activate signal transduction events in a specific cell region. This would lead to spatially restricted actin rearrangement and membrane protrusion in that area, although inhibiting signaling and preventing lamellar extension in another cell region. Supporting this view, raft dispersion at the leading lamella of growth cones, but not in other cell parts, impairs axon migration [14]. This

![Fig. 3. ‘Crafts’, a higher complexity level required for polarization. A series of events leading to gradient sensing and cell polarization after chemoattractant stimulation is shown. Cell polarization is initiated by chemoattractant interaction (spatial cue) with chemosensory receptors. This creates initial membrane asymmetry and triggers several signaling pathways (GTPases and PI3K) that lead to actin remodeling and raft domain rearrangement. Raft reorganization involves segregation of distinct lipid types to each cell pole; L-rafts are enriched in chemosensory receptors and U-rafts, in adhesion receptors. This unique compartmentalization characterizes leukocytes as bipolar sensors optimized for cell–cell (at the U-raft) and cell–chemoattractant (at the L-raft) interactions. L- and U-raft segregation to the leading edge and the uropod thus resemble the separation between the bow and stern of ‘crafts’. ‘Craft’ organization probably aids in establishing interrelated feedback loops among these components, leading to cell polarization and gradient sensing. Abbreviation: L-rafts, leading edge rafts; PI3K, phosphatidylinositol 3 kinase; U-rafts, uropod-rafts.](http://treimm.trends.com)
indicates that rafts regulate migration in a spatially defined manner.

Raft spatial control of cell migration reaches maximum complexity in leukocytes that display distinct raft-associated functions at the leading edge and at the uropod. L-rafts are involved mainly in gradient sensing, whereas U-rafts are implicated in cell–cell interactions. During chemoattractant-induced leukocyte polarization, rafts not only assemble into large-scale domains but also segregate to opposite cell poles, defining the ‘bow’ and ‘stern’ of the migrating cell [60]. The term ‘raft’ might thus be appropriate to denote this higher level of complexity. ‘Craft’ has been also suggested for yeast, indicating that this complexity is not restricted to leukocytes but probably occurs in many other cell types when they polarize.

Inhibition of chemoattractant receptor signaling, actin polymerization or PI3K activity results in the inability of cells to polarize and to reorganize rafts in response to an attractant. Disruption of raft domains also affects chemoattractant receptor signaling, actin polymerization and PI3K activity [15,47,48,58]. This suggests that these components form part of positive feedback loops that reinforce previous steps (Fig. 3). Cytoskeletal rearrangements are required for raft clustering but raft coalescence simultaneously controls actin cytoskeleton polymerization. Raft reorganization to ‘crafts’ also enables local activation of specific signaling pathways by recruiting or excluding intracellular partners, highlighting the role of lipid rafts in the control of cell spatial orientation. It is difficult at present to establish an unequivocal order of events leading to gradient sensing and cell polarization, but raft reorganization during chemotaxis is essential for organizing intracellular signaling and amplifying signals from the extracellular environment.

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