CHAPTER

Membrane Lipid Rafts and Their Role in Axon Guidance

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Abstract

The plasma membrane of cells contains a variety of lipid and protein molecules that are often segregated and heterogeneously distributed in microdomains. Lipid rafts represent a generalized concept of membrane microdomains that are enriched in cholesterol and sphingolipids and, characteristically, resistant to cold detergent extraction. Lipid rafts have recently received considerable attention because they are thought to be involved in many cellular functions, in particular, signal transduction for extracellular stimuli. Many of these functions are also intimately related to the processes involved in neural development, including neurotrophic factor signaling and synaptic plasticity. Recent studies from our lab and others have indicated an important role for lipid rafts in axonal growth and guidance. Specifically, our data show that lipid rafts on the plasma membrane provide platforms for spatial and temporal control of guidance signaling by extracellular cues. In addition, lipid rafts may also function in other aspects of axonal growth and guidance, including spatial and temporal regulation of adhesion, cytoskeletal dynamics, and growth cone motility. Further elucidating how membrane rafts are involved in guided axonal growth would provide important insights into the intricate signaling mechanisms underlying neuronal wiring, which is fundamental for normal brain development and functional recovery after injury and diseases.

Introduction

In the fluid mosaic model of the plasma membrane posited by Singer and Nichols, the membrane is a bilayer composed of a relatively continuous and homogenous fluid of amphipathic lipids that is interspersed with a mosaic of proteins.[1] Most eukaryotic cells are mainly composed of lipids belonging to three major lipid classes: glycerophospholipids, sphingolipids, and sterols. Various membrane proteins, including receptors, can associate with the plasma membrane by virtue of hydrophobic and electrostatic forces, covalently attached lipid anchors, and membrane-spanning domains. However, this picture of cell membranes has since been evolving steadily.[2] For example, it is known that the lipid and protein constituents of membranes are distributed asymmetrically. Different lipid classes of the membrane have been found in ratios that vary across each leaflet of the membrane, different cell types, and different cell compartments. The diversity of lipids and their distinct spatial distribution suggest that they may be involved in a variety of cellular functions. Arguably the most significant modification of the original fluid mosaic model is the existence of lipid domains of different lipid composition and physical state from the rest of the lipid bilayer. The initial notion of lipid domains was suggested by studies in model membranes, but it was the observation of caveolae, flask-shaped

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plasmalemmal invaginations of the cell membrane that led to extensive studies on membrane microdomains.[3] Caveolae exhibit several distinct features including a special lipid composition rich in cholesterol and sphingolipids, a striped coat formed by caveolin proteins on the cytoplasmic surface, and in addition to their characteristic flask shape, they can also have vesicular and tubular morphologies. Caveolae were initially thought to mainly function in clathrin-independent endocytosis.[3] Subsequent biochemical analyses of the molecular composition of caveolae, based on the findings that caveolae are low-density membranes and resistant to cold detergent extraction, suggested other possible functions. Most notably, these studies have found the presence of multiple signaling components in caveolae preparations,[3,4] indicating that caveolae may also play a role in signal transduction.

Later studies have pointed out that membrane domains lacking caveolin proteins are also present on the plasma membrane, suggesting the existence of other types of detergent-resistant microdomains (DRMs) that do not involve caveolins.[5] An increasing number of studies have now established that the plasma membrane contains different types of DRMs and caveolae represent a subset.[5] As such the term "lipid rafts" was later used to describe dynamic membrane domains in a broader sense. Before exploring the functions of rafts, it is helpful to consider some of their characteristics. Lipid rafts are small and dynamic: they can be as little as several nanometers in diameter and their transient existence is in the msec range. Both lipid raft size and half-life are flexible parameters that are altered in live cells, which may be involved in lipid raft functions. Rafts are thought to be a liquid ordered phase of membrane, which consists of saturated sphingolipids. The rafts "float" in a liquid disordered phase, which mainly consists of unsaturated glycerophospholipids. Cholesterol is thought to stabilize the sphingolipids in this liquid ordered phase since cholesterol interacts more favorably with sphingolipids over unsaturated phospholipids.[6] This partitioning of the membrane into laterally heterogeneous domains can therefore provide an organized membrane environment for protein interactions or other cellular functions.

Approaches to Study Lipid Rafts and Their Functions

Many studies have relied on two experimental approaches involving detergent resistance and cholesterol dependence to study lipid rafts and their functions. Lipid rafts are biochemically defined on the basis that they remain resistant to cold nonionic detergent treatment and/or are low-density membranes, thus float to the top of a buoyant density gradient. The so-named detergent resistant membranes (DRMs) are also known as detergent-insoluble glycolipid-enriched complexes (DIGs). Proteins that associate with lipid rafts are defined as those that cofractionate with DRM fractions and typically have some lipid modification such as glycosylphosphatidylinositol (GPI) or acyl anchors. Therefore, cold-detergent extraction and membrane fractionation have been extensively used to identify proteins associated with lipid rafts. Using this approach, numerous proteins, including GPI-anchored proteins, caveolins, src-family kinases, and G-proteins, have been shown to associate with lipid raft fractions.[7,8] Since lipid raft integrity depends on cholesterol, cellular functions that require lipid rafts could be affected by manipulating membrane cholesterol. Using various means to manipulate the synthesis or plasma member distribution of cholesterol has been instrumental in investigating the role of lipid rafts in cell functions beyond protein associations. While such experimental approaches have inherent flaws,[6,9] they have proved to be useful methods for identifying many of the constituents and functions involving lipid raft membrane microdomains.

One challenge in studying lipid rafts is the direct visualization of these dynamic microdomains on the native membrane of living cells.[10] While DRMs have been biochemically isolated and analyzed, the dynamics and spatial properties of lipid rafts remain to be directly examined. Detergent extraction does remove some lipids and proteins from rafts, which in combination with methodological differences, may account for the considerable degree of variability in analyses of raft components. However, it is the lack of visual evidence that is fueling the continuing debate over lipid rafts.[6] Past attempts of visualizing lipid rafts by light microscopy and electron microscopy have also generated conflicting results, particularly regarding the size of the rafts on

the cell membrane.[10-14] Perhaps the dynamic nature of these membrane domains and their spatial localization on the cell surface contribute to the difficulty in determining their size and distribution. Therefore, future improvement in spatial resolution of current microscopy techniques and the development of new imaging methods on living cells may finally reveal the spatial and temporal properties of DRMs. Among various promising techniques, high-resolution fluorescence resonance energy transfer (FRET) imaging offers the ability to study dynamic membrane microdomains and protein-protein interactions on the plasma membrane.[11,12,15] Such technical advances would allow the validation of the lipid rafts concept and our understanding of the dynamics and functions of these membrane microdomains.

Functions of Lipid Rafts in the Nervous System

Numerous functions of lipid rafts have been implicated in nonneuronal cells (for reviews, see refs. 7,8 and Fig. 1), many of which are likely involved in nerve cells. For example, the first of many functional roles attributed to membrane microdomains was in protein and lipid sorting in polarized epithelial cells. Considering that neurons are highly polarized cells with axonal and dendritic specifications, precise sorting and selective trafficking of different molecules are clearly required for the development and maintenance of specific structures and functions of the neuron. Moreover, membrane lipids and proteins are distributed with spatial differences at various locations of neurons. For example, dendritic spines have been shown to enrich sphingolipids and many postsynaptic proteins[16] while axonal processes contain specific molecules involved in motility and transmitter release, some of which have been shown to associate with membrane rafts.[17] While the exact mechanisms involved in the generation, regulation, and maintenance of neuronal polarity are still under investigation,[18,19] it is conceivable that lipid rafts may play an important role in sorting and trafficking of different molecules to specific neuronal locations, although further evidence is needed.

The notion that lipid rafts are involved in signal transduction was initially suggested by the cofractionation of many signaling components such as GPI-anchored proteins and src-family kinases with detergent resistant membranes.[9] Subsequent studies show that a variety of other receptors and intracellular signaling components are associated with DRMs. It was proposed that sphinglolipid-cholesterol microdomains act as rafts that can selectively associate with proteins and that such raft platforms are functionally involved in membrane trafficking and intracellular signaling.[20] These dynamic rafts are thought to provide suitable microenvironments, which in addition to enabling selective protein-protein interactions may also be involved in localized initiation of signal transduction.[7,8,21] Many different responses to extracellular signals by numerous cell types are currently thought to involve lipid rafts, including immune responses, growth factor signaling, adhesion, and chemotaxis. Importantly, these experiments indicate that lipid rafts can be involved in specific signaling pathways and/or other cell functions by distinct ways. Several models have been proposed on how rafts are involved in signaling, including resident or recruited signaling mechanisms.[8] Proteins with a high affinity for lipid rafts are generally thought to reside within lipid rafts, whereas other proteins with little or no affinity for lipid rafts can be recruited to rafts. Resident proteins associate with lipid rafts in the absence of a stimulus and typically include GPI-anchored proteins and dually acylated ones for the outer and inner leaflets of the plasma membrane, respectively. Even without such lipid modifications other proteins including transmembrane proteins may still reside in rafts by an unknown mechanism.

One of best examples on the role of lipid rafts in signal transduction is growth factor signaling (for review, see ref. 22). Recent evidence indicates that membrane rafts are involved in signaling induced by neurotrophins and glial cell line-derived neurotrophic factor (GDNF) families. The functions of these growth factor families can influence many different neuronal populations and include effects on cell growth, proliferation, differentiation, and survival. The signaling mechanisms that mediate the functions of these two families are similar in that they involve the activation of receptor tyrosine kinases, which leads to the formation of complexes that are coupled to multiple intracellular signaling events. However, signaling events induced by growth factor stimulation have been shown to involve lipid rafts in different ways.[22]

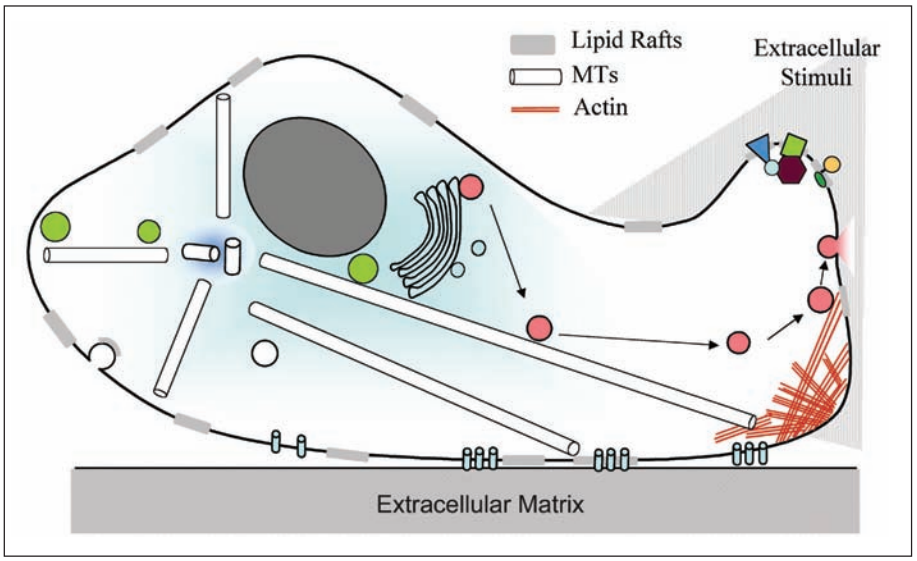

Figure 1. Functions of lipid rafts. This schematic view of a migrating cell (leading edge to the right) depicts the various functions that have been shown to involve lipid rafts. (1) Lipid rafts are involved in the sorting and trafficking of lipids and proteins to the plasma membrane of polarized cells. Vesicles budding from the Golgi are transported along microtubules to the front (red circles with thick border) or rear (green circles with thin border) of the cell. Certain types of endocytosis and exocytosis (unfilled vesicle and omega structure) also involve lipid rafts. It is possible that similar raft-dependent sorting methods are used by migrating cells to localize different sets of signaling components at the leading and trailing edges. (2) Many receptors and intracellular signaling molecules associate with lipid rafts and depend on these membrane microdomains for signal transduction events. Lipid rafts can serve as signaling platforms that enable the coupling of receptors to distinct pathways and can involve different variants of resident or recruited (induced association) mechanisms. (3) Regulation of the cytoskeleton is known to involve various proteins including the RhoGTPases as well as lipids such as certain phosphoinositides. Lipid rafts are thought to play a dynamic role in regulating the efficiency and membrane localization of these important protein and lipid regulators and thus the functions of the cytoskeleton. (4) Cell adhesion involves interactions between specific adhesion molecules on the cell with components of the extracellular matrix or other cells. Certain adhesion molecules associate with lipid rafts and their distribution on the cell may be regulated by lipid rafts and vice versa. It is important to consider that each lipid raft function can operate independent of the others during the overall functioning of the cell. It is also likely that some or all of the lipid raft functions operate in a coordinated fashion.

Neurotrophins, such as nerve growth factor (NGF), exert their effects by binding to receptor tyrosine kinase receptors (TrkA, B, and C) or a low-affinity receptor, p75NTR. Trk receptors and p75NTR have been found within lipid raft fractions along with critical intracellular components implicated in downstream signaling of these receptors. Evidence from PC12 cells shows that signaling from TrkA and p75NTR occurs and is enhanced within lipid rafts.[23] Thus neurotrophin binding to TrkA and p75NTR and subsequent signaling occurs within lipid rafts. In contrast to the involvement of rafts in neurotrophin signaling, evidence indicates that GDNF signaling involves recruitment of the receptor tyrosine kinase, c-Ret, to lipid rafts. It is thought that c-Ret recruitment to lipid rafts by GFRalpha enables this receptor tyrosine kinase to selectively associate with its downstream signaling components residing in lipid rafts.[24]

Neurotrophic factors have also been shown to be involved in synaptic plasticity. In particular, brain-derived neurotrophic factor (BDNF) is known to modulate long-term synaptic

potentiation. While accumulating evidence has indicated that lipid rafts can influence synaptic transmission through clustering and regulation of neurotransmitter receptors and affect the exocytotic process of transmitter release,[21] recent studies on BDNF effects have revealed some new insights towards how rafts may contribute to BDNF effects on synaptic plasticity.[25] For example, TrkB receptors were recruited to the raft fraction after exposure to BDNF and the translocation depended on tyrosine kinase activity. Furthermore, BDNF recruited TrkB alone into lipid rafts without carrying its associated proteins Shc, Grb2, and PLCγ, which is different from neuregulin-induced recruitment of ErbB4 to lipid rafts. Moreover, the finding that lipid rafts are only involved in BDNF modulation of synaptic activity, but not BDNF enhancement of neuronal survival indicates that these membrane rafts could be involved in signaling specificity of BDNF on developing neurons. Finally, the coreceptor p75 was found to inhibit BDNF-induced TrkB translocation into lipid rafts, suggesting that TrkB and p75 mediate distinct signaling pathways that depend differentially on lipid raft association. Future studies would likely elucidate the intermediate factors that interact with TrkB in the rafts for initiating specific downstream signaling leading to synaptic modification.

Membrane Domains and Growth Cone Motility

Lipid rafts have been implicated in many aspects of cell motility, in particular, cytoskeletal dynamics to substrate adhesion. Significantly, many of the molecular components regulating the actin cytoskeleton, cell motility, and adhesion are associated with rafts, including Rho GTPases, Src-family of tyrosine kinases, phosphoinositides PtdIns(4,5)P2 and PtdIns(3,4,5)P3.[26] In migrating cells, selective adhesion is established by the formation of focal adhesion complexes containing many signaling components and cytoskeletal anchoring. It is well established that cell migration requires dynamic and spatial regulation of focal adhesion complexes: adhesion at the rear end of the cells needs to be removed while the leading front forms new adhesion sites. The findings that distinct raft-associated components are asymmetrically distributed on the leading edge and the uropod suggest that lipid rafts are involved in the spatially-regulated motile activities in migrating cells. Recently, it has been shown that lipid rafts mediate signal transduction events initiated by cell adhesion to the extracellular matrix through integrins. In particular, membrane rafts appear to mediate spatial targeting of Rho GTPases to the plasma membrane for differential association with the downstream effectors for further signaling events, including Rac coupling to focal adhesion kinase and microtubule stabilization by Rho and mDia.[27,28] These findings from nonneuronal cells thus establish that lipid rafts play an important role in cell adhesion and motility by participating in signal transduction and spatial targeting of various signaling components.

In developing neurons, guided elongation of axonal processes depends critically on the motility and pathfinding ability of the tip of the axon, the growth cone for reaching the specific targets. Such directional motility is believed to depend on cytoskeletal dynamics, together with selective adhesion with the substratum, for steering the growth cone in response to a variety of environmental cues. Cell adhesion is mediated by interactions between the growth cone's cell adhesion molecules (CAMs) and the extracellular matrix or other CAMs on neighboring cells. Previous studies have shown that certain CAMs are associated with lipid rafts, indicating that selective adhesion underlying growth cone motility may involve lipid rafts. Recent studies have shown that nerve growth cones exhibit discrete lipid domains on the surface (Fig. 2, see also ref. 29) and raft disruption affected their motility on the adhesion molecule substrates L1, N-cadherin, but not β1 integrin.[29] These results show that growth cone adhesion on selective substrates involves lipid rafts. Furthermore, biochemical analyses have revealed that many other proteins involved in growth cone adhesion and motility are associated with DRMs, including focal adhesion kinases, src family of tyrosine kinases, GAP43, and etc.[17] Therefore, lipid rafts likely play a broader role in growth cone motility during migration. Since directed movement of cells or growth cones requires concerted events among the cytoskeleton, membrane anchoring, and cell-substratum adhesion, lipid rafts could serve as the central platforms for spatial and temporal regulation of any of these important events.

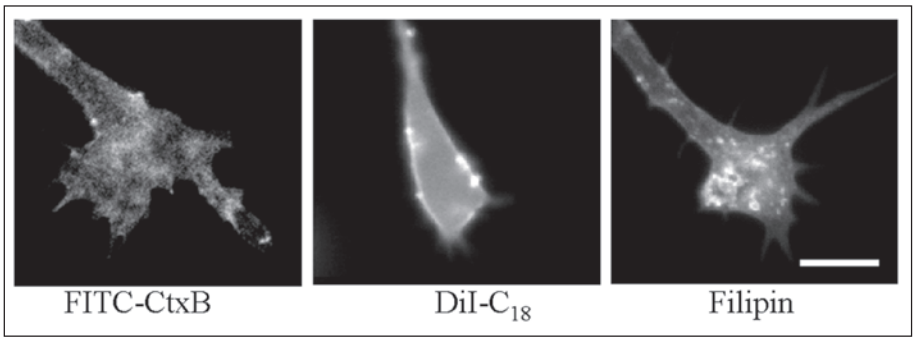

Figure 2. Lipid domains on the growth cone. The plasma membrane of *Xenopus* growth cones was stained with FITC-Cholera Toxin B (CTxB), $DiIC_{18}$, and filipin to examine the distribution of different lipid constituents. Staining of the membrane with the lipophilic dye, $DiIC_{18}$, resulted in a relatively uniform fluorescent signal. On the other hand, labeling of ganglioside G_{M1} by CTxB or cholesterol by filipin showed an apparent heterogeneous distribution, indicating the existence of microdomains. The image of FITC-CTxB staining was processed by applying a digital threshold for better illustration of domains. Scale bar = 10 μm.

Lipid Rafts in Axon Guidance: The Signaling Platforms?

Developing axons are guided to their targets by a variety of extracellular cues that either attract or repel growth cones. Many of these extracellular cues exert their specific actions on developing axons by binding to their surface receptors to initiate complex signaling cascades.[30-32] Therefore, the formation of ligand-receptor complexes on the plasma membrane represents the first step in transduction of guidance signals. In addition, many guidance cues elicit additional steps on and/or within the plasma membrane to generate distinct signaling cascades, including receptor oligomerization and complex formation with coreceptors and/or other membrane-associated components.[33] These protein interactions at/within the plasma membrane are believed to define distinct cellular responses to extracellular stimuli. For example, receptor cross-talk has been shown to specifically enable a particular guidance response while silencing the other.[34] While the membrane components involved in receptor-signaling complexes are being identified, how these receptors, coreceptors, and other membrane-associated components interact on the membrane to generate specific signaling cascades for distinct axonal responses remains elusive. The fact that these important events occur at or within the plasma membrane suggests that the membrane lipid environment could be crucial for the signal transduction of these extracellular guidance cues. On the other hand, specific lipid molecules are known to play an important role in cell signaling. For example, phospholipid phosphatidylinositol-3,4,5-trisphosphate (PIP_3) accumulates at the leading edge of chemotactic cells to recruit signaling proteins containing pleckstrin homology (PH) domains; such localization of PIP_3 at the leading edge is believed to be an essential part of directional responses of chemotactic cells.[35,36] These specific phospholipids (e.g., PIP_2 and PIP_3) may also depend on the lipid environment on the membrane for their localization and functioning.[37] Therefore, the specific lipid composition on the plasma membrane may contribute to not only protein-protein interactions but also lipid signaling in response to extracellular molecules.

So far, only a few guidance molecules are known to have an association with lipid rafts. For example, ephrin ligands and Eph receptor tyrosine kinases are well known molecules involved in axon guidance and topographic mapping of neuronal connections. Ephrin A proteins are GPI-anchored ligands residing in lipid rafts. Interestingly, ephrin B ligands contain a transmembrane domain, are also located in lipid rafts, and have been shown to use lipid rafts for signal transduction.[38] Previous studies have also shown that myelin-associated glycoproteins inhibit axonal growth through interactions with specific gangliosides and rafts.[39] Furthermore,

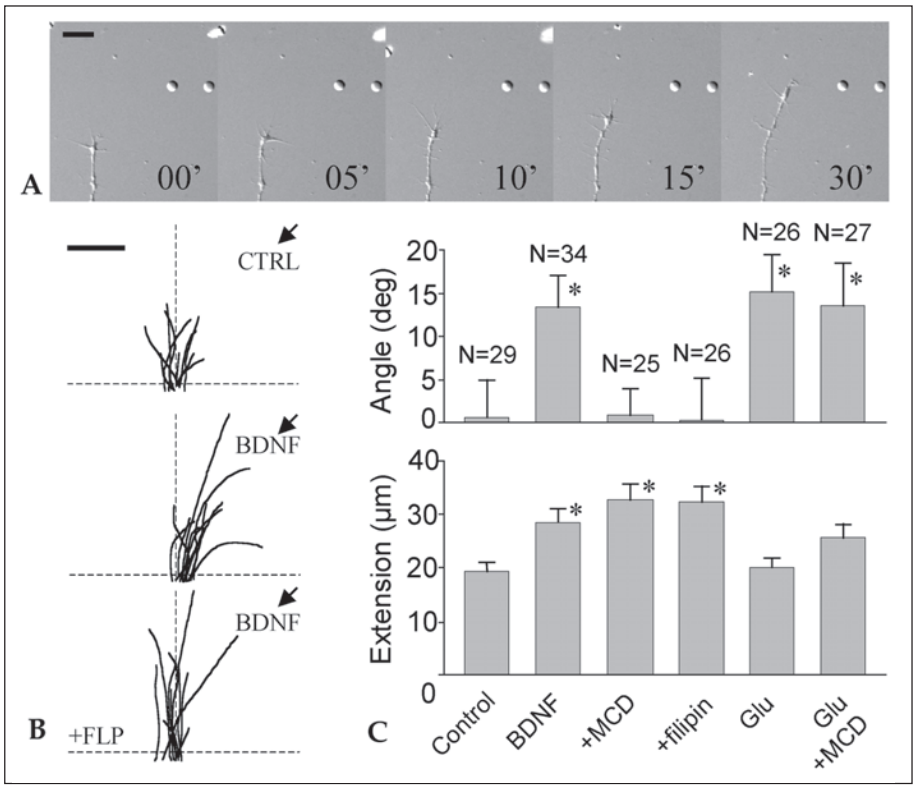

Figure 3. Lipid rafts are involved in functional guidance responses. The requirement of lipid rafts in functional guidance responses was evaluated in vitro by using the growth cone turning assay for the BDNF, netrin-1, Sema3A and glutamate as discussed in the text. We have included some raw data using BDNF as an example. A) Growth cone attraction to a gradient of BDNF is shown in the time lapse DIC images of an individual neurite. The numbers indicate the minutes since the onset of BDNF application. B) Superimposed traces of individual neurite trajectories during the assay period are shown for three experimental groups of growth cones. The origin represents the center of the growth cone that was extending vertical at the beginning of the assay. The arrow indicates the position of the pipette and the "+" sign indicates the addition of a raft disrupting agent. C) Growth cones were incubated with the indicated with two different agents that manipulate membrane cholesterol and thereby disrupting rafts. The growth cones were then exposed to a gradient of BDNF or glutamate to determine whether or not lipid rafts are involved in responses to these ligands. The bar graphs represent the average responses and indicate that lipid raft disruption blocks BDNF, but not glutamate attraction. Interestingly, raft disruption does not block growth promotion by BDNF. These results suggest that lipid rafts are selectively involved in certain functions, namely BDNF attraction, but not BDNF growth promotion, nor glutamate attraction. Scale bars: 20 μm.

NgR and p75NTR, the receptor and coreceptor for Nogo have been shown to reside in lipid rafts.[23,40,41] While these studies indicate the potential involvement of lipid rafts in guidance, they do not directly assess whether or not lipid rafts mediate signal transduction in guidance responses. Using an in vitro guidance assay together with several complementary methods of raft manipulation, we have recently shown that lipid rafts mediate guidance responses of nerve growth cones to BDNF, Netrin-1, and Sema3A gradients[42] (see also Fig. 3). The finding that activation of the MAPK p44/p42 by these guidance molecules could be abolished by raft

disruption suggests that lipid rafts are involved in signal transduction of these guidance responses. While the receptors for these ligands were only weakly associated with lipid rafts under control conditions, they increased their affinity with rafts in response to stimulation by the respective ligand. The mechanism responsible for this translocation and downstream events during growth cone guidance are not known. Recent studies on TrkB signaling indicate that activation of TrkB is a preceding requirement for localization into rafts, which specifically mediates BDNF modulation of synaptic transmission.[25] It is possible that association of ligand-receptor complexes within lipid rafts engages distinct signaling pathways from signaling outside rafts.[8,22,25] Growth cone guidance by BDNF and netrin-1 involves phospholipase C (PLC) and PI-3 kinases,[43] both of which associate with lipid rafts.[44] On the other hand, Sema3A signaling involves receptor complexes consisting of neuropillin-1, plexin A, and the adhesion molecule L1.[33,45] It is conceivable that, although all these guidance cues depend on rafts for their effects on growth cones, the specific signaling pathways could differentially rely on distinct raft-dependent mechanisms for generating guidance responses. It will therefore be important to determine whether these relevant signaling components associate with lipid rafts and the relative contributions of signaling in and out of rafts during the growth cone response.

Although we have shown that guidance signaling involves lipid rafts, the contribution of raft-dependent adhesion and/or cytoskeletal regulation in growth cone responses requires further investigation. Many nonreceptor tyrosine kinases involved in adhesion are associated with lipid rafts[8] and active Rho GTPases, which regulate the cytoskeleton, are targeted to lipid rafts for coupling to downstream effectors.[27,28,46] Moreover, cytoskeletal dynamics have been implicated in affecting the position and stability of rafts as well as the associations of certain molecules with rafts.[26] Therefore, lipid rafts may mediate growth cone guidance by providing a critical platform for coupling activated receptors, and/or their downstream effectors with the regulation of adhesion and the cytoskeleton. Rafts have also been implicated in organizing cellular polarity and as such they may be involved in the spatial localization of guidance signaling to mechanisms of adhesion and cytoskeletal regulation.[26] On the other hand, our findings that growth cone attraction induced by glutamate gradients was not affected by raft disruption indicate that lipid rafts were likely involved in signal transduction specific for BDNF, netrin-1, and Sema3A, rather than common steering events. Perhaps, different substrates may contribute to the raft-dependent and -independent adhesion and growth cone motility.[29]

Signal Localization through Lipid Rafts

Similar to chemotactic cells, growth cone turning in responses to guidance gradients involves asymmetric signaling. The recent finding that lipid rafts are functionally involved in such asymmetry provides an exciting avenue for pursuing the subcellular mechanisms of growth cone turning.[42] Some studies on polarization and asymmetric signaling in cell migration suggest that membrane receptors are not spatially redistributed in response to a chemotactic signal and that intracellular gradients are sufficient for encoding spatial information that mediates chemotactic responses.[36] Other studies suggest certain membrane components including receptors and lipid raft markers do exhibit a change of distribution during chemotaxis.[47] Consistent with this latter notion, there is evidence that the TrkB receptor asymmetrically associates with lipid rafts in response to the application of a BDNF gradient. Although the mechanism of this translocation is not known, such asymmetric localization of the receptor is thought to only occur at lipid rafts and would be sufficient to localize subsequent signaling steps required for turning. Furthermore, asymmetric translocation of guidance receptors into lipid rafts after ligand binding could lead to local signal amplification by concentrating signaling molecules and/or excluding unwanted modulatory components,[8] which might be essential for successful sensing of extracellular gradients. That raft subtypes asymmetrically redistribute during cell migration, suggests the exciting possibility that a similar mechanism may operate during growth cone guidance.

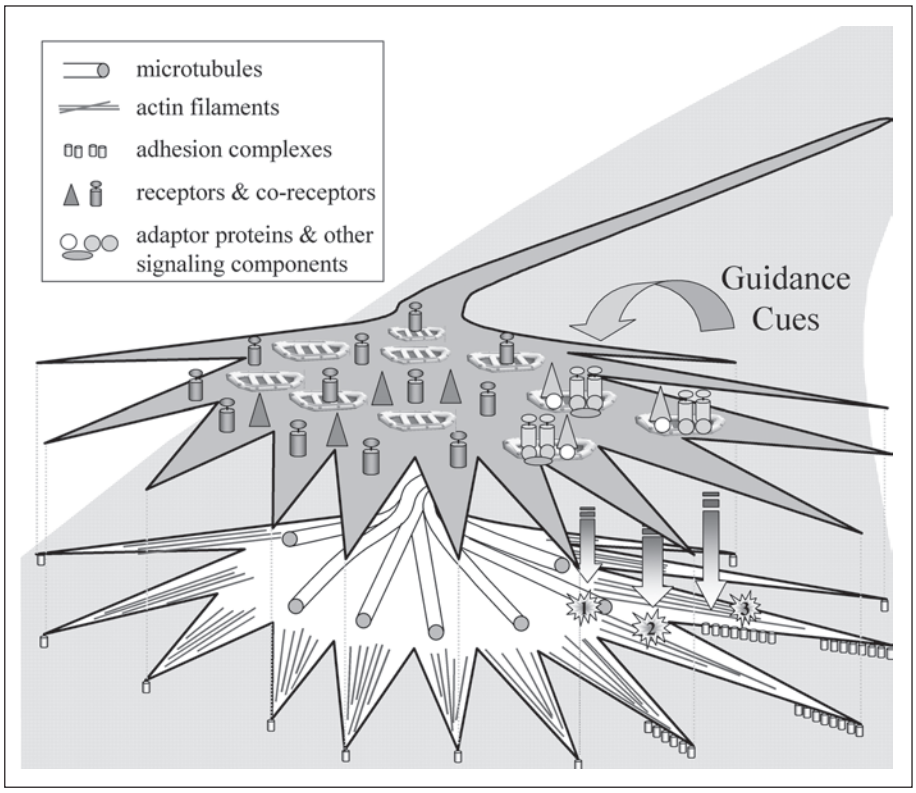

Figure 4. Hypothetical model on the role of lipid rafts in axon guidance and growth cone motility. We propose that lipid rafts provide critical platforms for spatial control of signaling in growth cone guidance to achieve asymmetric signal transduction and growth cone steering. Specifically, membrane receptors and certain raft types are likely to be distributed with relative uniformity on the plasma membrane in the absence of ligand stimulation. In response to stimulation by a guidance cue, the association of the appropriate receptor with lipid rafts increases, which could enable selective interactions with intermediate components residing in rafts for downstream signaling. Such an association may be maintained over time and can serve to amplify the stimulus in an asymmetrically activated signaling complex. Rafts could also be involved in local regulation of both cytoskeletal dynamics and adhesion for directional steering. Importantly, associations with rafts are dynamic events that can be regulated over time and space. For example, modulation of a guidance response may be achieved by regulating the affinity of a signaling component for lipid rafts and thus its ability to interact with other members of the complex. It also stands to reason that any process, which affects the positioning of lipid rafts, would be able to provide an overarching level in a hierarchy of membrane organization.

Based on the evidence discussed above, we propose a model in which lipid rafts provide critical platforms for spatial control of signaling in growth cone guidance to achieve asymmetric signal transduction and growth cone steering (Fig. 4). Lipid rafts can be involved in generating and/or maintaining the asymmetry at several steps along the signal transduction pathway. As the first step, ligand induced translocation of receptors to lipid rafts could enable selective interactions with intermediate components residing in rafts for downstream signaling. Such translocation could also function to shield the activated receptors from nonraft factors that can inactivate the receptors, thus providing a degree of temporal control. Furthermore, membrane

microdomains could also provide the platforms for specific targeting and formation of signaling complexes that enable the activation of selective pathways for distinct effects. Finally, rafts could be involved in local control of cytoskeletal dynamics and adhesion for directional steering. Ultimately, different guidance molecules and different environmental settings (e.g., different ECMs) could specifically utilize some of these raft-dependent mechanisms for spatiotemporal regulation of growth cone migration. The challenge would be to dissect the pathways and the specific functions of lipid rafts in each of the many guidance systems.

Future Directions and Concluding Remarks

The findings of a functional role of membrane rafts in axon guidance have also opened new directions for elucidating the molecular mechanisms underlying axon guidance and regeneration. For example, the findings on the ligand-induced translocation of receptors into lipid rafts suggest that coreceptors and/or intermediate signaling components are readily present in lipid rafts, waiting for activated receptors to signal downstream. Therefore, proteomic approaches could be used to analyze membrane raft factions with and without exposure to specific guidance cues, which could potentially lead to the identification of novel intermediate signaling components in the rafts.[48] Moreover, the observation that inhibitory effects of Semaphorin 3A could be abolished by raft manipulation also indicates a potential approach for overcoming inhibition of regenerating axons after nerve injury and diseases. Such an approach could represent a novel strategy for targeting axon inhibition during nerve regeneration and functional recovery. Finally, given the diverse roles potentially played by lipid rafts in various cellular functions, further studies are clearly required to delineate the specific steps of signaling transduction associated with distinct raft functions.

The cell's ability to sense and respond to environmental stimuli is crucial for many functions including neural development, immunity, angiogenesis, wound healing, and embryogenesis. Directed migration of nerve growth cones and chemotactic cells likely requires similar coordinated cellular processes that lead to movement, including sorting and trafficking of specific membrane proteins and lipids, signal amplification and localization, and spatiotemporal regulation of cytoskeletal dynamics and adhesion. Membrane microdomains may be of particular importance for migrating cells as they can serve to spatially and temporally coordinate the component functions required for cell and growth cone movement. Comparative analyses on the various roles and mechanisms related to lipid rafts in cell migration and growth cone guidance may be particularly helpful in understanding the precise functions of lipid rafts in intricate guidance signaling. These studies could in turn provide further mechanistic insights into directed cell migration underlying many important biological responses such as leukocyte chemotaxis during inflammatory response.

References

1. Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science 1972; 175(23):720-31.
2. Edidin M. Lipids on the frontier: A century of cell-membrane bilayers. Nat Rev Mol Cell Biol 2003; 4(5):414-8.
3. Anderson RG. The caveolae membrane system. Annu Rev Biochem 1998; 67:199-225.
4. Sargiacomo M, Sudol M, Tang Z et al. Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. J Cell Biol 1993; 122(4):789-807.
5. Harder T, Simons K. Caveolae, DIGs, and the dynamics of sphingolipid-cholesterol microdomains. Curr Opin Cell Biol 1997; 9(4):534-42.
6. Munro S. Lipid rafts: Elusive or illusive? Cell 2003; 115(4):377-88.
7. Brown DA, London E. Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 1998; 14:111-36.
8. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 2000; 1(1):31-9.
9. Edidin M. The state of lipid rafts: From model membranes to cells. Annu Rev Biophys Biomol Struct 2003; 32:257-83, (Epub 2003 Jan 16).
10. Lai EC. Lipid rafts make for slippery platforms. J Cell Biol 2003; 162(3):365-70.

11. Kenworthy AK, Petranova N, Edidin M. High-resolution FRET microscopy of cholera toxin B-subunit and GPI- anchored proteins in cell plasma membranes. Mol Biol Cell 2000; 11(5):1645-55.
12. Varma R, Mayor S. GPI-anchored proteins are organized in submicron domains at the cell surface. Nature 1998; 394(6695):798-801.
13. Glebov OO, Nichols BJ. Lipid raft proteins have a random distribution during localized activation of the T-cell receptor. Nat Cell Biol 2004; 6(3):238-43, (Epub 2004 Feb 8).
14. Pitto M, Palestini P, Ferraretto A et al. Dynamics of glycolipid domains in the plasma membrane of living cultured neurons, following protein kinase C activation: A study performed by excimer-formation imaging. Biochem J 1999; 344(Pt 1):177-84.
15. Sekar RB, Periasamy A. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. J Cell Biol 2003; 160(5):629-33.
16. Hering H, Lin CC, Sheng M. Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J Neurosci 2003; 23(8):3262-71.
17. He Q, Meiri KF. Isolation and characterization of detergent-resistant microdomains responsive to NCAM-mediated signaling from growth cones. Mol Cell Neurosci 2002; 19(1):18-31.
18. Winckler B, Mellman I. Neuronal polarity: Controlling the sorting and diffusion of membrane components. Neuron 1999; 23(4):637-40.
19. Foletti DL, Prekeris R, Scheller RH. Generation and maintenance of neuronal polarity: Mechanisms of transport and targeting. Neuron 1999; 23(4):641-4.
20. Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997; 387(6633):569-72.
21. Tsui-Pierchala BA, Encinas M, Milbrandt J et al. Lipid rafts in neuronal signaling and function. Trends Neurosci 2002; 25(8):412-7.
22. Paratcha G, Ibanez CF. Lipid rafts and the control of neurotrophic factor signaling in the nervous system: Variations on a theme. Curr Opin Neurobiol 2002; 12(5):542-9.
23. Huang CS, Zhou J, Feng AK et al. Nerve growth factor signaling in caveolae-like domains at the plasma membrane. J Biol Chem 1999; 274(51):36707-14.
24. Paratcha G, Ledda F, Baars L et al. Released GFRalpha1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts. Neuron 2001; 29(1):171-84.
25. Suzuki S, Numakawa T, Shimazu K et al. BDNF-induced recruitment of TrkB receptor into neuronal lipid rafts: Roles in synaptic modulation. J Cell Biol 2004; 167(6):1205-15. (Epub 2004 Dec 13).
26. Golub T, Wacha S, Caroni P. Spatial and temporal control of signaling through lipid rafts. Curr Opin Neurobiol 2004; 14(5):542-50.
27. del Pozo MA, Alderson NB, Kiosses WB et al. Integrins regulate Rac targeting by internalization of membrane domains. Science 2004; 303(5659):839-42.
28. Palazzo AF, Eng CH, Schlaepfer DD et al. Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. Science 2004; 303(5659):836-9.
29. Nakai Y, Kamiguchi H. Migration of nerve growth cones requires detergent-resistant membranes in a spatially defined and substrate-dependent manner. J Cell Biol 2002; 159(6):1097-108.
30. Tessier-Lavigne M, Goodman CS. The Molecular Biology of Axon Guidance. Science 1996; 274:1123-33.
31. Dickson BJ. Molecular mechanisms of axon guidance. Science 2002; 298(5600):1959-64.
32. Pasterkamp RJ, Kolodkin AL. Semaphorin junction: Making tracks toward neural connectivity. Curr Opin Neurobiol 2003; 13(1):79-89.
33. Huber AB, Kolodkin AL, Ginty DD et al. Signaling at the Growth Cone: Ligand-Receptor Complexes and the Control of Axon Growth and Guidance. Annu Rev Neurosci 2003; 28:28.
34. Stein E, Tessier-Lavigne M. Hierarchical organization of guidance receptors: Silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science 2001; 291(5510):1928-38.
35. Dekker LV, Segal AW. Perspectives: Signal transduction. Signals to move cells. Science 2000; 287(5455):982-3, 85.
36. Parent CA, Devreotes PN. A cell's sense of direction. Science 1999; 284(5415):765-70.
37. Laux T, Fukami K, Thelen M et al. GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. J Cell Biol 2000; 149(7):1455-72.
38. Bruckner K, Pablo Labrador J, Scheiffele P et al. EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. Neuron 1999; 22(3):511-24.
39. McKerracher L. Ganglioside rafts as MAG receptors that mediate blockade of axon growth. Proc Natl Acad Sci USA 2002; 99(12):7811-3.

40. Yu W, Guo W, Feng L. Segregation of Nogo66 receptors into lipid rafts in rat brain and inhibition of Nogo66 signaling by cholesterol depletion. FEBS Lett 2004; 577(1-2):87-92.
41. Higuchi H, Yamashita T, Yoshikawa H et al. PKA phosphorylates the p75 receptor and regulates its localization to lipid rafts. Embo J 2003; 22(8):1790-800.
42. Guirland C, Suzuki S, Kojima M et al. Lipid rafts mediate chemotropic guidance of nerve growth cones. Neuron 2004; 42(1):51-62.
43. Ming G, Song H, Berninger B et al. Phospholipase C-gamma and phosphoinositide 3-kinase mediate cytoplasmic signaling in nerve growth cone guidance. Neuron 1999; 23(1):139-48.
44. Inoue H, Miyaji M, Kosugi A et al. Lipid rafts as the signaling scaffold for NK cell activation: Tyrosine phosphorylation and association of LAT with phosphatidylinositol 3- kinase and phospholipase C-gamma following CD2 stimulation. Eur J Immunol 2002; 32(8):2188-98.
45. Castellani V, Rougon G. Control of semaphorin signaling. Curr Opin Neurobiol 2002; 12(5):532-41.
46. Michaely PA, Mineo C, Ying YS et al. Polarized distribution of endogenous Rac1 and RhoA at the cell surface. J Biol Chem 1999; 274(30):21430-6.
47. Gomez-Mouton C, Abad JL, Mira E et al. Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. Proc Natl Acad Sci USA 2001; 98(17):9642-7. Epub 2001 Aug 7.
48. Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci USA 2003; 100(10):5813-8. Epub 2003 Apr 30.