cellular signals, but how such a wide variety of signals is generated and how they work in combination is unknown. For example, Netrin stimulates changes in intracellular calcium, phospholipase C, phosphatidylinositol 3-kinase, mitogen-activated protein kinase, and the small GTPases Cdc42 and Rac1 (reviewed in Guan and Rao, 2003). While some of these signals certainly modulate the growth cone cytoskeleton directly, others likely affect distinct cellular processes such as protein synthesis and degradation, as well as vesicle trafficking and ion channel activity. These additional effects may ultimately feedback to amplify or modulate the intracellular signals generated by receptor activation. Given the complexity of signaling networks that exists for individual guidance cues, it is bewildering to imagine how growth cones in vivo integrate signals generated by simultaneous activation of multiple receptors.

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Selected Reading

Bashaw, G.J., Kidd, T., Murray, D., Pawson, T., and Goodman, C.S. (2000). Cell *101*, 703–715.

Bear, J.E., Svitkina, T.M., Krause, M., Schafer, D.A., Loureiro, J.J., Strasser, G.A., Maly, I.V., Chaga, O.Y., Cooper, J.A., Borisy, G.G., and Gertler, F.B. (2002). Cell *109*, 509–521.

Colavita, A., and Culotti, J.G. (1998). Dev. Biol. *194*, 72–85.

Coppolino, M.G., Krause, M., Hagendorff, P., Monner, D.A., Trimble, W., Grinstein, S., Wehland, J., and Sechi, A.S. (2001). J. Cell Sci. *114*, 4307–4318.

Gitai, Z., Yu, T.W., Lundquist, E.A., Tessier-Lavigne, M., and Bargmann, C.I. (2003). Neuron *37*, 53–65.

Guan, K.L., and Rao, Y. (2003). Nat. Rev. Neurosci. *4*, 941–956.

Hong, M., Lee, P.N., Pang, K., Byrum, C.A., Bince, J.M., Xu, R., Martindale, M.Q., and Nishiyama, M. (2003). Nature *426*, 446–450.

Kao, H.T., Song, H.J., Porton, B., Ming, G.L., Hoh, J., Abraham, M., Czernik, A.J., Pieribone, V.A., Poo, M.M., and Greengard, P. (2002). Nat. Neurosci. *5*, 431–437.

Lebrand, C., Dent, E.W., Strasser, G.A., Lanier, L.M., Krause, M., Svitkina, T.M., Borisy, G.G., and Gertler, F.B. (2004). Neuron *42*, this issue, 37–49.

Machesky, L.M. (2000). Cell *101*, 685–688.

Reinhard, M., Jarchau, T., and Walter, U. (2001). Trends Biochem. Sci. *26*, 243–249.

Song, H.J., and Poo, M.M. (2001). Nat. Cell Biol. *23*, 81–88.

Svitkina, T.M., Bulanova, E.A., Chaga, O.Y., Vignjevic, D.M., Kojima, S., Vasiliev, J.M., and Borisy, G.G. (2003). J. Cell Biol. *160*, 409–421.

Lipid Rafts as Organizing Platforms for Cell Chemotaxis and Axon Guidance

Lipid rafts are thought to serve as plasma membrane platforms for localized trafficking and signaling. Recent findings reported by Guirland et al. in this issue of *Neuron* and by Gómez-Moutón in a recent issue of *JCB* support a direct role of lipid microdomains in organizing spatial signaling during axon guidance and cell chemotaxis by concentrating the gradient-sensing machinery at the leading edge.

The existence of discontinuous microdomains in the plasma membrane of eukaryotic cells has been a topic of intense debate in recent years. Discrete plasma membrane domains with different properties could be important for targeting specific components to different locations in the cell and for compartmentalization of signaling pathways. As such, they could contribute to a variety of important biological processes, including endo- and exocytosis, signal transduction, cell polarity, antigen recognition, cell adhesion and migration, axon guidance, and synapse formation and function. The notion that specific lipids, particularly cholesterol and sphingolipids, could serve to organize membranes into distinct microdomains has gained support from studies using model lipid bilayers, detergent extraction, cholesterol depletion, and examination of the cellular distribution of glycosylphosphatidylinositol (GPI)-anchored proteins, widely regarded as markers of such domains. Lipid microdomains are envisioned as discrete platforms of a particular lipid and protein composition floating in an otherwise uniform sea of plasma membrane, and they are therefore usually called lipid rafts. Although their mere existence remains a contentious issue (see Simons and Toomre, 2000, and Munro, 2003, for recent reviews on either side of the controversy), the concept of lipid rafts continues to inspire a great number of researchers to examine how compartmentalization and clustering of different types of molecules in the plasma membrane may contribute to downstream signaling and cell behavior. Among the voluminous literature on lipid rafts (over 1000 Medline hits, 97% from the past 5 years), two recent papers, one of them in this issue of *Neuron*, stand out for their quality and elegance in providing some of the first direct evidence of the role of lipid rafts in organizing spatial signaling during axon guidance and cell chemotaxis (Gómez-Moutón et al., 2004; Guirland et al., 2004 [this issue of *Neuron*]).

In their study in this issue of *Neuron*, Guirland et al. (2004) took advantage of the growth cone turning assay first developed by Mu-ming Poo and colleagues (Lohof et al., 1992) to examine the role of lipid rafts in the chemotropic guidance of axons. In this assay, growth cones on a dish are confronted with chemotropic substances emanating from a micropipette placed at a fixed distance and angle. Chemoattractants make growth cones turn toward the pipette, while chemorepellents deflect growth cones away from it. In their experiments, Guirland et al. found that the chemoattractant effect of a diffusible gradient of brain-derived neurotrophic factor (BDNF) could be eliminated upon disruption of lipid rafts by membrane cholesterol depletion or by treatment with the cholesterol-sequestering agent filipin or with the ganglioside G_{M1}, which perturbs raft stability. Although cholesterol depletion has been shown to have a number of effects on the overall integrity of the plasma membrane, including the release of certain protein compo-

nents, Guirland et al. showed that growth cone turning responses in cholesterol-depleted axons could be rescued by subsequent addition of low concentrations of cholesterol, in agreement with a reversible effect of drug treatment on lipid rafts. Interestingly, neither cholesterol depletion nor sequestration had any effect on the net growth rate of axons, which in the presence of BDNF was consistently increased compared to control conditions. Thus, lipid rafts would appear to have a specific role in growth cone turning responses to a diffusible gradient of BDNF but not in the overall ability of this neurotrophin to promote axonal extension. In contrast, however, treatment with G_{M1} abolished BDNF-induced axonal growth in addition to blocking turning responses, indicating possible effects of this ganglioside on BDNF signaling events within the growth cone. In fact, an interaction between gangliosides and neurotrophin signaling has been reported in a number of experimental paradigms (Ferrari et al., 1995; Pitto et al., 1998), suggesting that growth cones may not be able to appropriately respond to BDNF in the presence of G_{M1}. The importance of lipid raft integrity for growth cone guidance was not limited to chemoattraction, as growth cone repulsion by gradients of netrin-1 or Semaphorin 3A was also blocked after disruption of lipid rafts.

So far so good, but the literature is already abundant in examples of loss-of-function effects as a result of cholesterol depletion. What makes the study of Guirland et al. particularly special is their ability to distinguish between general or permissive versus direct or instructive roles of lipid rafts in guiding the directional responses of axons. Growth cone turning in response to diffusible gradients of chemotropic substances is likely to involve asymmetric activation of membrane receptors and localized intracellular signaling. Guirland et al. reasoned that if lipid rafts have a direct role in the spatial organization of asymmetric signaling in the growth cone, then localized disruption of lipid rafts on one side of the growth cone should also create an asymmetry in receptor activation in axons growing in the presence of uniform concentrations of guidance cues. Importantly, that asymmetry should be of the opposite direction to that created by a gradient of the same guidance cue. That is exactly what they observed after focal application of the cholesterol-depleting agent methyl-β-cyclodextrin (MCD) on one side of growth cones growing in an homogenous concentration of BDNF. Thus, simply reverting the mode of application of the two substances resulted in the repulsion of growth cones away from the pipette containing MCD. Of note, focal application of MCD had no effect on growth cone turning in the absence of BDNF, indicating that MCD was not per se acting as a repellent in this assay. Moreover, growth cones could be made attracted to the pipette containing MCD by allowing them to grow in a uniform concentration of netrin-1, thus ruling out unspecific or toxic effects of focal application of MCD on turning responses.

These elegant results clearly demonstrate that local signaling via lipid rafts mediates growth cone turning responses to chemotropic cues. However, how do they do it? Conceivably, lipid rafts could simply potentiate receptor activation in response to ligand. On the other hand, they could have a more direct role by organizing asymmetric signaling across the growth cone in response to gradients of chemotropic substances. More specifically, what is the spatial relationship between lipid rafts and the receptors for guidance cues? Is this altered in any way during turning responses? Guirland et al. found that ligand treatment increased the levels of both the BDNF receptor TrkB and the netrin-1 receptor DCC in detergent-resistant membranes, a biochemical surrogate of lipid rafts, and induced copatching between antibody-clustered receptors and lipid raft aggregates produced by clustering of G_{M1} gangliosides with the cholera toxin B subunit. Thus, the relevant guidance receptors appeared to be recruited to lipid rafts, at least as detected by these two methods. But what about the turning? In their final experiment, Guirland et al. imaged TrkB and G_{M1} gangliosides in situ in detergent-resistant membranes of growth cones before and after exposure to a diffusible gradient of BDNF. The results showed that BDNF induced an asymmetric distribution of TrkB receptors that colocalized with G_{M1} on the side of the growth cone exposed to the chemotropic factor. Although arguably a bit rough, the experiment does suggest that lipid rafts play a role in the spatial organization of signaling platforms containing chemotropic receptors, and probably other downstream components, within turning growth cones.

This result is important, as it provides evidence for a role of lipid rafts in mediating growth cone turning that is not based on the use of cholesterol-depleting drugs (but, alas, still relying on detergents), and as such warrants future efforts for further refinement. For example, novel FRET techniques (FRET stands for fluorescence resonance energy transfer) have been used to monitor protein-protein interactions within lipid rafts in intact cells. Recently, using cells expressing fluorescent GPI-anchored proteins, Sharma and colleagues applied homo- and hetero-FRET methods together with theoretical modeling to study the size and distribution of cholesterol-dependent lipid microdomains in living cells (Sharma et al., 2004). They found that about 20%–40% of the GPI-anchored proteins formed very small ($<$5 nm) cholesterol-sensitive clusters, each composed of at most 4 molecules. These nanoscale clusters are arguably as close as we have come so far of an objective definition of lipid microdomains. Due to their nonperturbing nature, sensitivity, and resolution, FRET-based methods are likely to become the technology of choice in future studies of lipid-dependent raft organization.

Another issue that would need to be addressed is whether gradients of guidance cues induce an asymmetric distribution of lipid rafts in the living growth cone. This could be done by live imaging of cells expressing fluorescent GPI-anchored proteins as recently demonstrated by Gómez-Moutón and colleagues in their elegant study on the role of lipid microdomains during leukocyte chemotaxis (Gómez-Moutón et al., 2004). These researchers had previously shown that depletion of plasma membrane cholesterol-inhibited cell polarization and migration in response to chemoattractants. They had also observed that during chemotaxis, raft domains distributed asymmetrically in the cell, with G_{M3}-containing rafts enriched at the leading edge and G_{M1}-containing rafts at the rear or uropod, while chemoattractant receptors accumulated only at the leading edge (Mañes et al., 1999; Gómez-Moutón et al., 2001). These results had raised the possibility that lipid rafts may act as signal amplification centers during cell polarization

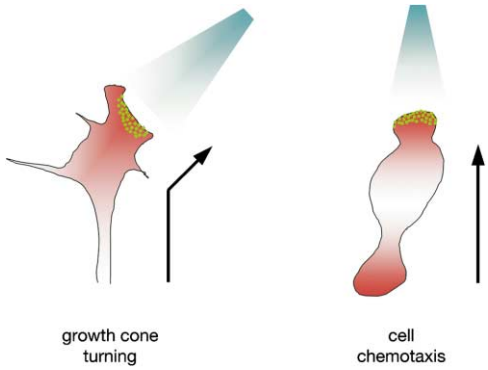

Figure 1. Spatial Organization of Signaling by Lipid Rafts

Lipid rafts (red) organize spatial signaling during growth cone guidance (left) and cell chemotaxis (right) by concentrating the gradient-sensing machinery (green dots) at the leading edge. Diffusible gradients are shown in blue.

and chemotaxis, but since the evidence for asymmetric raft distribution was obtained in fixed cells, it was not known whether rafts in fact redistribute during directional cell movement in living cells. In the new work, they used high-resolution confocal video microscopy to track raft-associated fluorescent GPI-anchored proteins during leukocyte chemotaxis in response to a diffusible gradient of the chemokine stromal cell-derived factor-1 (SDF-1). They found that chemoattractants induced a persistent redistribution of raft-associated GPI-anchored green fluorescent protein (GFP) to both cell edges during cell chemotaxis, while transmembrane, nonraft proteins were distributed homogeneously. In agreement with the results of Guirland et al., they also found that the chemokine receptor CCR5 accumulated in GM3-rich rafts at the leading edge. They also observed recruitment and activation of phosphatidylinositol-3 kinase γ (PI3Kγ) in leading edge rafts of migrating cells. As expected, cholesterol depletion prevented raft redistribution and asymmetric recruitment of PI3Kγ.

Together, the results of Guirland et al. and Gómez-Moutón et al. indicate that lipid rafts organize spatial signaling in moving cells and growth cones by concentrating the gradient-sensing machinery at the leading edge (Figure 1). In contrast, a recent study concluded that lipid raft proteins have a random distribution during focal activation of T cell receptors with coated beads in stationary T cells (Glebov and Nichols, 2004). Intriguingly, although these researchers did observe redistribution of fluorescent GPI-anchored proteins and G_{M1} gangliosides to the site of activation, in agreement with previous work in that field (Viola et al., 1999), they failed to detect significant changes in surface density of GPI-linked proteins using FRET (Glebov and Nichols, 2004). They therefore suggested that the apparent local concentration of lipid raft components seen in some experiments could be explained by convolution of the plasma membrane to generate an increase in fluorescence intensity. Although that could be the case of static cell membranes in direct contact with beads coated with stimulating substances, it would seem less likely in the case of a growth cone moving toward or away from a diffusible gradient of a chemotropic guidance cue. Moreover, as observed by Gómez-Moutón et al., the fact that nonraft transmembrane proteins distributed homogeneously in cells undergoing chemotaxis would argue in favor of an active mechanism of raft redistribution rather than membrane flow to the cell poles. A more exciting possibility suggested by the studies of Guirland et al. and Gómez-Moutón et al. is the idea that redistribution of lipid raft components may be specifically important for directional cell or growth cone movement and perhaps less so for interactions between stationary components. Thus, lipid raft mobilization and the ensuing localized downstream signaling may be part of an intrinsic three-dimensional cellular response to spatial cues, a sort of "subcellular patterning" that allows the cell to spatially integrate environmental cues coming from specific directions. This type of mechanism could be of importance in a number of other processes involving dynamic subcellular polarization of some sort, such as axonal branching and asymmetric cell division.

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Selected Reading

Ferrari, G., Anderson, B.L., Stephens, R.M., Kaplan, D.R., and Greene, L.A. (1995). J. Biol. Chem. *270*, 3074–3080.

Glebov, O.O., and Nichols, B.J. (2004). Nat. Cell Biol. *6*, 6–243.

Gómez-Moutón, C., Abad, J.L., Mira, E., Lacalle, R.A., Gallardo, E., Jimenez-Baranda, S., Illa, I., Bernad, A., Mañes, S., and Martinez, A.C. (2001). Proc. Natl. Acad. Sci. USA *98*, 9642–9647.

Gómez-Moutón, C., Lacalle, R.A., Mira, E., Jimenez-Baranda, S., Barber, D.F., Carrera, A.C., Martinez, A.C., and Mañes, S. (2004). J. Cell Biol. *164*, 759–768.

Guirland, C., Suzuki, S., Kojima, M., Lu, B., and Zheng, J.Q. (2004). Neuron *42*, this issue, 51–62.

Lohof, A.M., Quillan, M., Dan, Y., and Poo, M.M. (1992). J. Neurosci. *12*, 1253–1261.

Mañes, S., Mira, E., Gomez-Mouton, C., Lacalle, R.A., Keller, P., Labrador, J.P., and Martinez, A.C. (1999). EMBO J. *18*, 6211–6220.

Munro, S. (2003). Cell *115*, 377–388.

Pitto, M., Mutoh, T., Kuriyama, M., Ferraretto, A., Palestini, P., and Masserini, M. (1998). FEBS Lett. *439*, 93–96.

Sharma, P., Varma, R., Sarasij, R.C., Ira, Gousset, K., Krishnamoorthy, G., Rao, M., and Mayor, S. (2004). Cell *116*, 577–589.

Simons, K., and Toomre, D. (2000). Nat. Rev. Mol. Cell Biol. *1*, 31–39.

Viola, A., Schroeder, S., Sakakibara, Y., and Lanzavecchia, A. (1999). Science *283*, 680–682.

Retina versus Cortex: Contrast Adaptation in Parallel Visual Pathways

Human vision adapts to the contrast of patterns by changing its sensitivity, but the origins of this perceptual adaptation have been disputed. In this issue of