



Review

Lipid rafts, cholesterol, and the brain

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ABSTRACT

Lipid rafts are specialized membrane microdomains that serve as organizing centers for assembly of signaling molecules, influence membrane fluidity and trafficking of membrane proteins, and regulate different cellular processes such as neurotransmission and receptor trafficking. In this article, we provide an overview of current methods for studying lipid rafts and models for how lipid rafts might form and function. Next, we propose a potential mechanism for regulating lipid rafts in the brain via local control of cholesterol biosynthesis by neurotrophins and their receptors. Finally, we discuss evidence that altered cholesterol metabolism and/or lipid rafts play a critical role in the pathophysiology of multiple CNS disorders, including Smith–Lemli–Opitz syndrome, Huntington's, Alzheimer's, and Niemann–Pick Type C diseases.

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1. Introduction

The role of cholesterol in cellular function has come under intense scrutiny with the advent of the lipid raft hypothesis (Simons and Ikonen, 1997, 2000; Maxfield and Tabas, 2005; Michel and Bakovic, 2007). Lipid rafts are commonly defined as cholesterol- and sphingolipid-enriched membrane microdomains that function as platforms that concentrate and segregate proteins within the plane of the bilayer (Simons and Ikonen, 1997; Pike, 2006). For the purposes of this discussion, we define caveolae as a subset of lipid rafts characterized by the presence of caveolin (Fielding, 2001). Since the advent of the raft hypothesis, the list of putative raft-mediated events has grown at a rapid pace. Initially proposed to function primarily in protein sorting events in polarized cells, lipid rafts are now thought to regulate membrane trafficking in both the exocytic and endocytic pathways, cell migration, and a variety of cell signaling cascades (Brown and London, 1998; Simons and Toomre, 2000). Lipid–lipid interactions are thought to be of fundamental importance to the formation of lipid rafts, with cholesterol playing a special role as the “glue” that holds these domains together (Barenholz, 2002). Cell membranes are thought to contain both raft and non-raft domains, and proteins in turn preferentially

associate with one type of domain or the other. Thus, the presence of lipid rafts is thought to enhance certain protein–protein interactions (for proteins contained within the same raft for example) while inhibiting others (by segregating raft and non-raft proteins). The specialized lipid composition of lipid rafts, characterized by an enrichment of cholesterol and sphingolipids, may also influence protein function directly by modulating the properties of the membrane bilayer.

Although the human brain only accounts for about 2% of total body weight, it contains as much as 25% of cholesterol and cholesterol derivatives (Dietschy and Turley, 2001, 2004). Given the abundance of cholesterol in the brain, it is only natural to ask what function lipid rafts play in the CNS. Lipid rafts are proposed to be critical for normal functioning of the brain and are found in both neuronal and glial cells (Tsui–Pierchala et al., 2002; Gielen et al., 2006; Debruin and Harauz, 2007). Lipid rafts serve as sites where the regulated intramembrane proteolysis of many transmembrane proteins takes place, including amyloid precursor protein, ErbB4, deleted in colorectal cancer, Delta 1 and Jagged 2, and p75 neurotrophin receptor (Landman and Kim, 2004; Vetrivel et al., 2005). Other proposed functions of lipid rafts in the nervous system include neuronal signaling, neuronal cell adhesion, and axon guidance (Paratcha and Ibanez, 2002; Golub et al., 2004; Kamiguchi, 2006; Michel and Bakovic, 2007). The presence of various ionotropic receptors and neurotransmitter transporters within rafts suggests a critical role of rafts in controlling neurotransmission (Tillman and Cascio, 2003). In this regard, cholesterol content of

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the rafts has a potential to effectively control the ion conductance and excitability of membranes, trafficking of ionotropic receptors to and from the cell surface, size and number of some postsynaptic receptor clusters (such as NMDA and GABA_A), and neurotransmitter signaling through G-protein coupled receptors (Stetzkowski-Marden et al., 2006; Willmann et al., 2006; Besshoh et al., 2007; Huang et al., 2007).

Lipid rafts consist of both protein and lipid components and are thought to exist in continuity with non-raft regions of membrane. This has made their identification and characterization particularly challenging. As such, many details of the structural properties of lipid rafts, and indeed their very existence, are hotly debated (Munro, 2003; Hancock, 2006; Jacobson et al., 2007). We thus begin our discussion with an overview of methods currently used to study lipid rafts as well as models for how lipid rafts form and function. Next, we outline a potential mechanism for regulating lipid rafts in the brain via local control of cholesterol biosynthesis by neurotrophins and their receptors. Finally, we discuss the consequences of deficits in cholesterol production/metabolism on human brain function, which may be linked to alterations in lipid raft function (Moebius et al., 2000; Nwokoro et al., 2001; Maxfield and Tabas, 2005).

2. Methods for studying lipid rafts in cells and artificial membranes

The earliest results supporting the lipid raft model used a biochemical approach to isolate a detergent-resistant membrane (DRM) fraction from polarized epithelial cells. This fraction was enriched in (glyco)sphingolipids, cholesterol, and glycosylphosphatidylinositol (GPI)-anchored proteins (Brown and Rose, 1992). Subsequent studies developed non-detergent based raft isolation methods (reviewed in Pike, 2003). Biochemical fractionation, either with or without detergent, remains by far the most widely used technique to isolate lipid rafts and their associated proteins (Brown, 2006). By this criterion, lipid rafts are abundant at the cell surface, enriched in cholesterol and sphingolipids, and contain a variety of proteins. A number of sorting signals that target proteins to DRMs have been identified. These include binding to raft-associated lipids such as sphingolipids or cholesterol, specific regions of the transmembrane domain, GPI-anchors, and other lipid modifications of peripheral membrane proteins such palmitoylation and myristoylation (Brown, 2006). Moreover, the association of proteins with lipid rafts can either be constitutive or transient (Lucero and Robbins, 2004).

Lipid–lipid interactions are one of the major mechanisms thought to underlie lipid raft formation (Simons and Ikonen, 1997; Rietveld and Simons, 1998; Simons and Vaz, 2004). In particular, DRMs are thought to represent liquid ordered (L_o) domains, which coexist in the same membrane with liquid disordered (L_d) domains (Schroeder et al., 1994; Brown and London, 1998). L_o domains form due to the preferential interactions of sphingolipids and/or phospholipids containing saturated acyl chains with cholesterol (Brown and London, 1998; Rietveld and Simons, 1998). In this state, lipids are tightly packed such that their order is close to that observed in the gel state, yet can undergo rapid lateral diffusion (Brown and London, 1998). The characteristic features of liquid ordered domains, as well as the phase behavior of mixtures of liquid ordered and liquid disordered domains, can be easily studied in simple mixtures of purified lipids (London, 2002; Silvius, 2003; Veatch and Keller, 2005). A great attraction of these artificial membrane systems is that the domains that form are often micron-sized in scale and thus can be directly visualized with fluorescent probes that show a preference for one phase (Brown, 2001). In addition, their composition can be readily manipulated, enabling studies of the effects of natural sterols, sterol derivatives, and

cholesterol precursors on liquid ordered domain formation (Xu and London, 2000; Xu et al., 2001; Megha et al., 2006).

Since cholesterol is required for the formation of L_o domains, removal of cholesterol from membranes would likewise be expected to cause the loss of lipid rafts (Simons and Toomre, 2000; Edidin, 2003; Pike, 2003). Thus, cholesterol depletion has also become widely used as an assay to study lipid rafts. In cells, cholesterol depletion can be accomplished through sequestration of cholesterol by cholesterol-binding compounds, removal of cholesterol from the membrane using methyl- β -cyclodextrin, and/or inhibition of cholesterol biosynthesis. Consistent with cholesterol's role in enabling lipid raft formation, cholesterol depletion often causes a loss of raft proteins from DRMs. In addition, cholesterol depletion has also been used in functional assays; pathways that are inhibited by cholesterol depletion are defined as raft-dependent (Simons and Toomre, 2000; Edidin, 2003; Pike, 2003).

Although biochemical fractionation and cholesterol depletion remain among the most widely used techniques to define the raft-associated proteins and the function of rafts in cells, a number of questions have been raised about the validity of these approaches (Munro, 2003; Shogomori and Brown, 2003; Lichtenberg et al., 2005; Zidovetzki and Levitan, 2007). In some instances, clustering of raft proteins in microdomains can be detected by electron microscopy. The best-studied examples include Ras, influenza hemagglutinin, and components of the high affinity IgE receptor signaling pathway (Wilson et al., 2002, 2004; Parton and Hancock, 2004; Hess et al., 2005; Takeda et al., 2003). In addition, large-scale lipid phase separation can be observed in plasma membrane blebs (Baumgart et al., 2007). Although these approaches have provided valuable information about lipid rafts and related membrane microdomains, their use is limited to fixed or perturbed cells. In living cells, lipid rafts have been exceptionally difficult to visualize.

Due to the difficulties of directly imaging lipid rafts in live cell membranes, biophysical techniques sensitive to protein and lipid dynamics and domain organization have instead been used to explore their properties (Fig. 1). Such techniques can be used to monitor raft-related events with high spatial and temporal resolutions without perturbing the integrity of the membrane (Lommerse et al., 2004; Jacobson et al., 2007). One such approach is fluorescence (or Förster) resonance energy transfer (FRET), used to test the hypothesis that raft proteins or lipids are enriched in domains with sub-micron dimensions (reviewed in Kenworthy, 2002; Lommerse et al., 2004; Lagerholm et al., 2005; Rao and Mayor, 2005; Silvius and Nabi, 2006). In this technique, proteins or lipids of interest are labeled with fluorophores known as a donor and acceptor. FRET occurs when the donor- and acceptor-labeled molecules are within ~ 100 Å of one another. The extent of FRET is quantified in terms of the efficiency of energy transfer. This is typically detected by monitoring changes in the fluorescence of the donor fluorophores in the presence or absence of the acceptor or by looking for emission of the acceptor when exciting at the donor wavelength. Alternatively, it can be monitored by following changes in the polarization of fluorescence under conditions where homoFRET, i.e. FRET in which the same type of fluorophore serves as both donor and acceptor, is measured.

Importantly, the efficiency of FRET is very strongly distance dependent, falling off as the inverse of the 6th power of the distance between the labeled molecules. Thus, FRET provides a convenient way to determine the proximity of two molecules to one another – for example, their colocalization within the same lipid raft – over distances smaller than that could be measured by conventional fluorescence microscopy (Fig. 1). For proteins and lipids in membranes, FRET can occur by chance if the concentration of donors and acceptors is high enough. However, it is possible to distinguish this type of “non-specific” FRET from FRET that occurs as the result of clustering of proteins within lipid rafts, as well as to

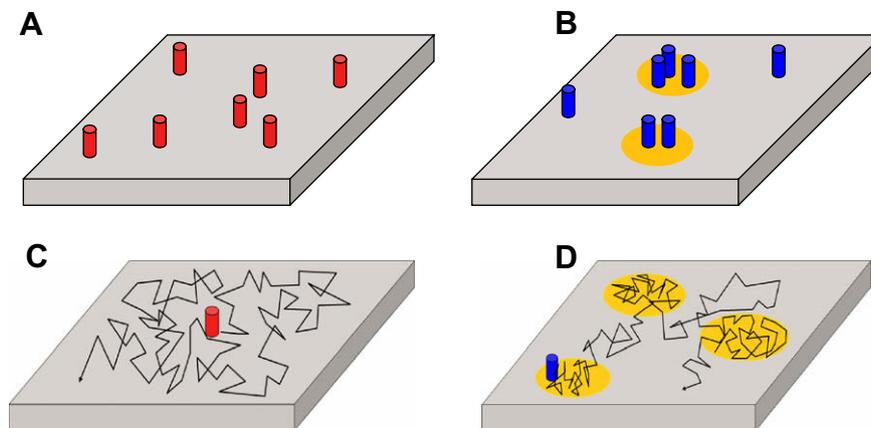


Fig. 1. Properties of lipid rafts that can be probed by FRET (A, B) and diffusion-based measurements (C, D). Bulk membrane is depicted in gray, lipid rafts in yellow, raft-associated proteins in blue, and non-raft proteins in red. The movement of proteins by diffusion within the plane of the membrane is indicated by the black lines. (A): In the absence of lipid rafts, a random protein distribution is expected. This gives rise to either no FRET or a FRET signal that shows a characteristic dependence on the surface density of labeled proteins. A similar result would be expected for proteins that are neither enriched nor excluded from lipid rafts; (B): the association of proteins with lipid rafts brings them within FRET proximity. For FRET to be able to detect such domains, at least one donor-labeled and one acceptor-labeled protein must localize within the same lipid raft; (C): for proteins undergoing classical free diffusion, diffusion is not hindered in any way; (D): one way that lipid rafts can impact protein diffusion is by transiently confining them to liquid ordered domains. This can be detected experimentally by testing for cholesterol-dependent, confined diffusion.

test various models of domain organization, with the aid of mathematical modeling (Kenworthy and Edidin, 1998; Sharma et al., 2004; Kiskowski and Kenworthy, 2007).

Based on FRET criteria, some raft proteins show little evidence for clustering in FRET studies (Kenworthy and Edidin, 1998; Kenworthy et al., 2000; Glebov and Nichols, 2004), while others appear to be clustered in sub-micron domains, as predicted by the lipid raft model (Varma and Mayor, 1998; Zacharias et al., 2002; Nichols, 2003; Sharma et al., 2004). Interestingly, detailed analysis of such FRET data has shown that only a small fraction of GPI-anchored proteins is found in clusters and that these clusters consist of only a few molecules (Sharma et al., 2004). These findings raise the possibility that raft proteins spend much of their time in a monomeric state and that the fraction of proteins associated with lipid rafts may be highly regulated. It remains to be determined if this type of domain organization is specific to GPI-anchored proteins or a common feature of various types of raft-associated proteins. How the fraction of proteins associated with lipid rafts is regulated also is currently unknown. Further studies on the sub-micron distribution of additional raft proteins should help to resolve these issues. Finally, in addition to provide information about the size and area fraction of lipid rafts, FRET also holds great potential to reveal transient interactions between raft-associated molecules, as illustrated by a recent FRET study of dynamic events that occur during B-cell signaling (Sohn et al., 2006).

Lipid rafts have also been hypothesized to impact the ability of membrane proteins to diffuse freely within the plane of the membrane (Kenworthy, 2005; Fig. 1). For example, protein diffusion could be slowed within the specialized liquid ordered environment of lipid rafts. Other models suggest that proteins may be transiently associated with lipid rafts, especially if the rafts are crosslinked (Dietrich et al., 2002; Shvartsman et al., 2003; Chen et al., 2006b). Alternatively, proteins may diffuse as part of a raft complex (Pralle et al., 2000). These effects have been studied using techniques sensitive to lateral diffusion such as fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS), and single molecule tracking (reviewed in Lommerse et al., 2004; Kenworthy, 2005; Lagerholm et al., 2005).

FRAP measures the diffusion of a population of fluorescently labeled molecules by monitoring the recovery of fluorescence into a small region after subjecting it to a rapid bleaching event, which occurs via diffusional exchange of the bleached molecules with

fluorescent molecules in the surrounding region by diffusion (Chen et al., 2006a). The speed of recovery is characterized by a diffusion coefficient (units of length²/time, typically cm²/s or μm²/s), and the extent of recovery provides a measure of the fraction of molecules that are free to diffuse or mobile fraction. Differences in these parameters between raft and non-raft proteins, or changes as the result of cholesterol depletion, are used to probe for the association of proteins with lipid rafts versus non-raft domains (Niv et al., 2002; Kenworthy et al., 2004). On the basis of such experiments, caveolae have been shown to be quite stable and immobile (van Deurs et al., 2003). On the other hand, under steady state conditions, raft proteins can diffuse relatively rapidly, suggesting lipid rafts are dynamic structures (Kenworthy et al., 2004). Interestingly, in polarized epithelial cells, the diffusion of non-raft proteins, but not raft proteins, is highly temperature dependent (Meder et al., 2006). This suggests that the plasma membrane of these cells consists of dispersed non-raft domains separated by a connected raft phase at low temperatures and, conversely, dispersed raft domains separated by connected non-raft domains at 37 °C (Meder et al., 2006). However, because FRAP is a population-based measurement, it cannot provide detailed information about the mechanisms that lead to such changes in either bulk diffusion coefficients or mobile fractions at the level of single molecules.

Single particle tracking follows the behavior of individual protein and lipid molecules and thus yields insights into the ways in which their diffusion is regulated. Here, very sensitive detectors are required to enable the visualization of single molecules and tracing of their movements. Interpretation of the resulting trajectories also requires considerable analysis (Ritchie and Kusumi, 2003; Chen et al., 2006a). Nevertheless the outcomes of such experiments can be extremely informative. One question that can be addressed, for example, is whether the relative diffusion of a pair of proteins is correlated in a manner consistent with their confinement to rapidly diffusing, small domains (Vrljic et al., 2002). Other single particle tracking studies have addressed the question of how GPI-anchored proteins, a class of raft-associated molecules, behave upon cross-linking by multivalent antibodies, a stimulus known to induce intracellular signaling pathways by poorly understood mechanisms (Chen et al., 2006b; Suzuki et al., 2007a,b). Impressively, such studies allow one to trace out the signaling pathways involved by monitoring the recruitment of individual signaling proteins to sites of crosslinking. Protein diffusion during T-cell signaling, another

example of a signaling event thought to involve lipid rafts, has also been investigated using single molecule techniques. This approach revealed an unexpected role of protein–protein networks in regulating the enrichment or exclusion of proteins from sites of T-cell signaling (Douglass and Vale, 2005).

FCS monitors the movement of small numbers of fluorescent molecules through a very small observation volume (Elson, 2004). Autocorrelation analysis of the resulting fluorescence fluctuations over time provides information about the number of molecules passing through the volume and their characteristic residence time, which is related to their diffusion coefficient. In the lipid raft field, FCS has proven to be especially useful in studies in artificial membranes, but can also be applied to cells (Kahya and Schwill, 2006). A variation on FCS called cross correlation jointly monitors the movement of two different colors of fluorescent molecules simultaneously. Using this technique, it is possible to test for cross-correlations indicative of molecule diffusing together as a complex. This type of analysis holds great potential for elucidating molecular interactions that occur during events such as cell signaling mediated by raft-associated proteins and lipids (Larson et al., 2005).

In addition to these approaches, new methods are also being brought to bear to study lipid rafts in cells, including techniques sensitive to membrane ordering (Gaus et al., 2006; Davey et al., 2007) and superresolution techniques such as fluorescence photoactivation localization microscopy that enable localization of molecules with sub-diffraction precision (Hess et al., 2007). Thus, the arsenal of biophysical tools to investigate lipid rafts and related membrane domains is continuing to grow.

3. Molecular mechanisms underlying raft formation and function

Lipid rafts are hypothesized to function by a process of compartmentalization (Simons and Ikonen, 1997; Pierce, 2002; Manes et al., 2003; Pike, 2003; Parton and Hancock, 2004). In this model, interactions of molecules within the same raft are enhanced, while interactions of non-raft and raft molecules are inhibited due to their spatial segregation.

But, how does segregation of proteins into raft and non-raft domains occur? As already introduced, the most commonly cited model suggests that lipid rafts correspond to L_0 domains that coexist with L_d domains. In turn, different raft proteins exhibit varying “affinities” for rafts, reflected by the extent to which they associate with L_0 domains (Brown, 2006). To test this idea, recent *in vitro* studies have begun to examine the partitioning of peptides and/or proteins into L_0 versus L_d domains (Hammond et al., 2005; Vidal and McIntosh, 2005). However, partitioning is not the only mechanism by which lateral compartmentalization of proteins could be achieved, and it is not yet clear that this is the most appropriate model to describe the behavior of raft proteins and lipids in cell membranes. For example, some proteins expected to associate with L_0 domains on the basis of their association with DRMs in cells show little partitioning when studied in liposomes (Shogomori et al., 2005). With the recent development of methods to examine lipid domain segregation in plasma membrane blebs, it should be possible to begin to examine this model in more detail (Baumgart et al., 2007; Sengupta et al., 2008).

Other models have focused on explaining how rafts can be functional if they are small and transient structures. One of these, the lipid shell model, suggests that individual “raft” proteins are surrounded by a shell of raft lipids, giving rise to their characteristic association with detergent-resistant membrane fractions (Anderson and Jacobson, 2002; Jacobson et al., 2007). The lipid shell model allows for the existence of raft proteins as monomers, yet also suggests the shells could function to target proteins to pre-existing domains/stable domains such as caveolae, or form

larger domains via regulated self-assembly and/or crosslinking. Yet another model is suggested by the observations that the clustering of certain raft proteins occurs in a concentration-independent fashion (Sharma et al., 2004). This indicates that the fraction of proteins associated with domains is constant over a wide range of expression levels, a result that implies these domains are actively regulated by the cell. The possibility that protein–protein or protein–lipid interactions stabilize small transient domains and/or induce the formation of larger, longer-lived entities has been proposed by a number of groups (Kusumi et al., 2004; Kusumi and Suzuki, 2005; Hancock, 2006; Jacobson et al., 2007). Finally, the possibility that cell membranes exist close to a phase boundary and thus small changes in lipid composition could drive raft assembly or disassembly has also been suggested (Keller et al., 2004; Veatch and Keller, 2005).

To date, most studies have focused on the role of cholesterol in driving the formation of lipid rafts. It is clear, however, that the presence of cholesterol in lipid rafts should have physical consequences on the membrane as well, by influencing membrane thickness, elasticity, and even curvature (Allende et al., 2004; Bacia et al., 2005). These structural consequences of cholesterol enrichment may in turn impact the ability of certain proteins to associate with lipid rafts, as illustrated by studies of the sorting of peptides between liquid ordered and liquid disordered domains *in vitro* (McIntosh et al., 2003). Protein activity may additionally be modulated by the specialized lipid environment of rafts (Pike, 2003).

In summary, current models differ in their proposed details of the molecular mechanisms underlying lipid raft formation. Nevertheless, most models agree that cholesterol is a critical regulator of lipid raft function. Thus, local regulation of cholesterol levels via control of cholesterol biosynthesis could represent a powerful mechanism to control raft-dependent events such as signaling and trafficking. Below, we discuss evidence that such a regulatory mechanism may in fact occur in the brain, through a feedback loop that links regulation of the cholesterol biosynthesis machinery and growth factor function.

4. Regulation of CNS cholesterol biosynthesis: a novel mechanism for regulation of lipid rafts?

Cholesterol plays a wide variety of roles in the CNS, most notably as a precursor for steroid hormones and myelin, in addition to its potential role in forming lipid rafts (Simons and Ikonen, 2000; Dietschy and Turley, 2001; Fielding, 2001). Cholesterol biosynthesis starts with acetyl-CoA as a substrate and involves at least 20 enzymes (Dietschy and Turley, 2001, 2004). The rate-limiting enzyme in cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Hmgcr; EC 2.3.3.10) which catalyzes conversion of Hmg-CoA to mevalonic acid. In addition to cholesterol biosynthesis, the cholesterol pathway plays a pivotal role in the synthesis of many bioactive compounds, including four key intermediates: mevalonate, farnesyl pyrophosphate, squalene, and lanosterol. Mevalonate is the first committed intermediate in the synthesis of dolichols that are carriers for addition of carbohydrate chains to glycoproteins. Farnesyl pyrophosphate is an important precursor for nonsterol isoprenoids geranylgeranyl pyrophosphate, heme A, and ubiquinone. The first dedicated step in *de novo* cholesterol biosynthesis begins with formation of squalene and ends with the reduction of 7-dehydrocholesterol by 7-dehydrocholesterol reductase (Dhcr7; EC 1.3.1.21) into cholesterol (Bae et al., 1999).

All of brain cholesterol is synthesized locally, with the highest rate of synthesis occurring during first postnatal weeks in humans and rodents (Jurevics and Morell, 1995; Jurevics et al., 1997), and this time window corresponds to the peak of myelination process. Myelin, produced by oligodendrocytes, ensheathes axons and is

critical for the conduction of action potential along the neurons (Miller, 2002). It has high lipid and cholesterol contents, which are critical for its insulating function. Disturbances in lipid homeostasis result in altered CNS structure and function: deficient cholesterol biosynthesis in oligodendrocytes delays myelination (Saher et al., 2005), while altered biosynthesis of other lipids (e.g. galactolipids) gives rise to defects in myelination (Marcus and Popko, 2002). These findings indicate that development and maintenance of myelin membranes require a complex interplay of lipids and proteins, and membrane rafts may play a critical role in this process (Gielen et al., 2006; Debruin and Harauz, 2007; Colognato et al., 2002).

While there is a clear role of oligodendrocytes in myelin formation, the respective roles of neurons and glial cells in cholesterol biosynthesis in the adult nervous system are poorly understood (Pfrieger, 2003). Although the prevailing view is that in the adult nervous system astrocytes produce brain-derived cholesterol (Pfrieger, 2003), there have been suggestions that neuronal cells, in addition to glial cells, are also capable of cholesterol synthesis (Saito et al., 1987; Suzuki et al., 2007c). A detailed map of the expression of cholesterologenic enzymes in the adult nervous system is not yet available. However, our recent study revealed that adult cortical, cholinergic and hippocampal neurons express high transcript levels of Hmgcr and Dhcr7 (Korade et al., 2007). The co-expression of the first and last enzymes of the cholesterologenic pathway in cortical, hippocampal, and cholinergic neurons (Korade et al., 2007) suggests that these neurons may require high levels of endogenous cholesterol production for their normal function. There are also regional differences in the distribution profile of cholesterol-synthesizing enzymes (Ness, 1994; Bae et al., 1999), sterol-sensing factors, intracellular transporters, lipoprotein receptors, and cholesterol shuttle proteins (Stockinger et al., 1998; Ong et al., 2000, 2004). Considering the complexity of adult nervous system, it is not surprising that different brain regions show marked differences in cholesterol content (Zhang et al., 1996). In particular, hippocampal and cortical neurons show different intrinsic concentrations of cholesterol (Ko et al., 2005). This difference is also present in the detergent-resistant membranes, suggesting that hippocampal neurons are also enriched in membrane rafts. Furthermore, the data suggest that cholesterol is a potent regulator of *in vitro* neuronal morphology: increased cholesterol levels lead to less complex dendritic arborization (Ko et al., 2005). Support for this view also comes from an independent study by Pooler et al. (2006), in which they report that an Hmgcr inhibitor, pravastatin, enhanced neurite outgrowth, neurite length, and neurite branching in cultured hippocampal neurons.

In addition to building evidence for regional differences in cholesterologenic enzyme expression, several recent lines of evidence suggest that their expression may be dynamically regulated by neurotrophic factors. Neurotrophic factors exert multiple biological functions in the nervous system. They are essential for the development of the brain; they promote survival and differentiation and are important for maintenance of synaptic contacts (Chao, 2003). A recent study by Suzuki et al. (2007c) showed that cortical and hippocampal neurons grown in culture for several days increase amount of cholesterol and this is due to increased expression level of cholesterol-synthesizing enzymes. Importantly, these investigators found that brain-derived neurotrophic factor (BDNF) regulates expression of cholesterologenic enzymes specifically in neurons but not in glial cells. Furthermore, this *de novo* synthesized cholesterol was incorporated into lipid rafts and BDNF stimulation lead to increased level of caveolin-2 and presynaptic proteins in rafts but not in non-rafts. The neurotrophic effects on the cholesterol/lipid raft system appear to be mediated through both the specific (TrkB) (Suzuki et al., 2007c) and common (p75) neurotrophin receptors (Korade et al., 2007). Blocking TrkB

prevented the increase in cholesterol (Suzuki et al., 2007c). Our recent studies revealed that p75 is a potent regulator of cholesterologenic enzyme expression in neuronal cultures and several human neuroblastoma cell lines (Korade et al., 2007). Overexpression of GDNF in neuronal progenitor cells was shown to regulate four enzymes of the cholesterol biosynthesis pathway but it is not known if these changes lead to alterations of lipid rafts (Pahnke et al., 2004). Similarly, microarray analysis of NGF-induced gene expression in PC12 cells revealed changes in cholesterol biosynthesis enzymes (Lee et al., 2005).

All of these studies revealed that neurotrophins and their receptors are involved in regulation of cholesterol metabolism. A simplified model of this regulation is shown in Fig. 2. Binding of neurotrophins leads to activation of their respective receptors and transcriptional regulation of lipidogenic enzymes in the nervous tissue. The altered lipid biosynthesis will lead to altered organization/composition of lipid rafts and changes in neurotransmitter release, cell division, and neuronal outgrowth. The growth factor–membrane raft interaction is a two-way street. Not only do growth factors regulate membrane raft structure, but membrane rafts are also essential for growth factor signaling. Growth factor receptor tyrosine kinases EGFR, Trk and PDGFR are enriched in lipid rafts and other receptors, normally located outside the rafts (e.g. Ret), can be recruited to the raft by GPI-anchored coreceptors (Paratcha and Ibanez, 2002; Tsui-Pierchala et al., 2002; Kamiguchi, 2006). Therefore, disruption of rafts is likely to impair signaling via these systems. Much more work is needed to define the consequence of this altered cholesterol biosynthesis on lipid raft composition and function.

5. Human conditions with altered cholesterol biosynthesis: consequences of raft dysfunction?

Cholesterol is not only important for normal brain function but also has been linked to CNS pathophysiology. Cholesterol disturbances in the CNS appear to lead to brain abnormalities

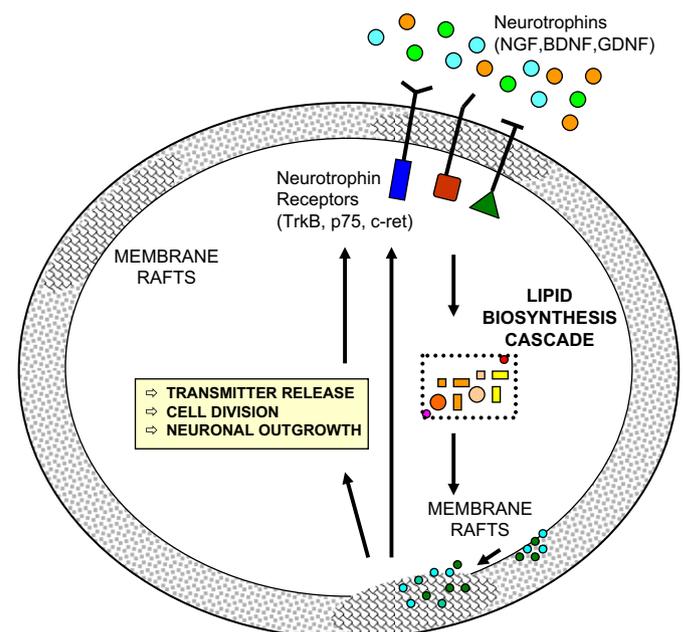


Fig. 2. Proposed feed-forward and feedback regulation of the lipid raft–neurotrophin signaling cascade. Binding of neurotrophins leads to activation of their respective receptors and transcriptional regulation of lipidogenic enzymes in the nervous tissue. The altered lipid biosynthesis will lead to altered organization/composition of lipid rafts and changes in neurotransmitter release, cell division, and neuronal outgrowth. Finally, altered microdomain structure will also affect the insertion and signaling of the neurotrophin receptors, thus completing the lipid raft/neurotrophin regulatory cycle.

(microcephaly, macrocephaly and mental retardation). Here, we discuss evidence that altered cholesterol metabolism and/or lipid rafts play a critical role in the pathophysiology of multiple CNS disorders, including Smith–Lemli–Opitz syndrome (Jira et al., 2003), Huntington's (Valenza et al., 2005), Alzheimer's (Wellington, 2004; Wolozin, 2004), and Niemann–Pick Type C (Vance et al., 2005) diseases. However, at the present time it is unclear which of these disturbances are mediated through raft-dependent mechanisms.

Reduced or absent Dhcr7 leads to Smith–Lemli–Opitz syndrome (SLOS), which is characterized by developmental deformities, incomplete myelination, and mental retardation (Jira et al., 2003). In SLOS, the tissue cholesterol and total sterol levels are markedly reduced while the concentrations of 7-dehydrocholesterol are greatly elevated (Jira et al., 2003). This elevation in 7-dehydrocholesterol inhibits the activity of Hmgcr, thus further exacerbating the cholesterol deficit (Honda et al., 1998; Fitzky et al., 2001). Importantly, as 7-dehydrocholesterol can incorporate into lipid rafts and alters the protein composition within them (Keller et al., 2004), at least some of the disturbances seen in SLOS can be due to the altered function of the lipid rafts. The greatly increased level of 7-dehydrocholesterol leads to increased membrane fluidity and decreased intermolecular packing of phospholipids fatty acyl chains (Vainio et al., 2006; Rog et al., 2007; Samuli Ollila et al., 2007). Membrane X-ray diffraction studies suggest that the difference between the structure of cholesterol and 7-dehydrocholesterol leads to significant differences in membrane lipid organization and dynamics (Xu et al., 2001). 7-Dehydrocholesterol differs from cholesterol in a double bond at the 7th position in the sterol ring, and this appears to be an important structural difference that translates into a functional difference: depletion of cholesterol from hippocampal membranes and replenishment with 7-dehydrocholesterol do not restore ligand-binding activity of the serotonin 1A receptor, despite the recovery of the overall membrane order (Singh et al., 2007). Based on the clinical symptoms of SLOS and our own findings of high neuronal expression of cholesterologenic enzymes (Korade et al., 2007), we propose that altered neuronal synthesis of cholesterol may be an important contributing factor to the SLOS pathophysiology, and that these events may involve altered membrane raft events.

In Huntington's disease, the expanded glutamate tract in the huntingtin protein leads to reduced cholesterol biosynthesis and neuronal cell death (Valenza et al., 2005). Specifically, the expression level of cholesterol biosynthesis genes was downregulated in human postmortem striatum and cortex as well as in brain tissue from HD mouse (Valenza et al., 2005). Interestingly, the application of exogenous cholesterol to striatal neurons expressing mutant huntingtin prevented their death (Valenza et al., 2005). The reduction of both cholesterol and BDNF in HD brains suggests a causal relationship between BDNF signaling and cholesterol homeostasis, and it is likely that one of the critical sites of disturbance is the signal transmission at the lipid rafts (Zuccato et al., 2005).

Alzheimer's disease is characterized by accumulation of amyloid beta peptide ($A\beta_{1-42}$) derived by presenilin 1/ γ secretase cleavage of amyloid precursor protein (APP) (Price et al., 1998; Sisodia and St George-Hyslop, 2002). Cholesterol metabolism is altered in the brain of patients with AD and the critical evidence of the importance of normal cholesterol homeostasis in AD comes from epidemiological studies (Wellington, 2004). These revealed that the prevalence of diagnosed AD is reduced in individuals taking statins (inhibitors of cholesterol biosynthesis) for long time periods (Wolozin, 2004). Experimental evidence revealed that statins are capable of reducing amyloid β_{42} deposition in animal models of AD (Refolo et al., 2001). The analysis of temporal cortex from AD subjects showed loss of lipid rafts (Molander-Melin et al., 2005), while hippocampi showed reduced cholesterol content in

the rafts (Ledesma et al., 2003). In addition the inhibition of cholesterol transport alters PS1 localization and APP processing in neuronal cells (Runz et al., 2002).

Finally, Niemann–Pick Type C (NPC) disease is characterized by accumulation of unesterified cholesterol and other lipids in endosomal vesicles, which leads to a set of CNS abnormalities that share clinical and histopathological similarities between NPC and AD: progressive dementia, neurofibrillary tangles, tau hyperphosphorylation, increased APP and abnormal accumulation of $A\beta_{42}$ in the hippocampus and cerebral cortex (Vance, 2006). Cultured striatal neurons from NPC KO mice do not respond to BDNF although they express TrkB on their cell membrane (Henderson et al., 2000). This is demonstrated by the absence of autophosphorylation of TrkB receptor and defective neurite outgrowth in NPC KO neurons (Henderson et al., 2000). This study corroborates the link between neurotrophins and cholesterol. The common clinical symptoms between AD and NPC suggest a set of shared pathophysiological events, and these most likely involve the cholesterol biosynthesis/lipid raft signaling system.

6. Perspectives

In the nervous tissue various molecular complexes are assembled in lipid rafts, thus mediating a diversity of downstream cellular events such as neurotransmitter processing and release, cell division, cell adhesion, and neuronal outgrowth. Most prominently, neurotrophin receptors are embedded in lipid rafts and BDNF, NGF and GDNF signaling depends on the normal functioning of lipid membrane microdomains. However, it appears that the lipid raft–neurotrophin interaction undergoes a dual feed-forward and feedback regulation: recent evidence suggests that the neurotrophin system is capable of regulating the lipid and cholesterol content of the rafts themselves. These findings, combined with evidence of altered lipid biosynthesis and neurotrophin disturbances in multiple CNS disorders, suggest that lipid rafts are likely to play a critical role in the pathophysiology of multiple CNS disorders, including Smith–Lemli–Opitz syndrome, Huntington's, Alzheimer's, and Niemann–Pick Type C diseases. Novel methods to study lipid rafts will aid in resolving composition of rafts and their role in nervous system function.

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