LIPID RAFTS AND NEURODEGENERATION: STRUCTURAL AND FUNCTIONAL ROLES IN PHYSIOLOGIC AGING AND NEURODEGENERATIVE DISEASES

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Abbreviations: Ganglioside and glycosphingolipid nomenclature is in accordance with the IUPAC-IUBMB recommendations (1). ABCA1, ATP binding cassette transported A1; AD, Alzheimer's disease; AICD, APP intracellular C terminus domain; APP, amyloid precursor protein; Cav1, caveolin 1; Cav3, caveolin 3; CGT, UDP-galactose ceramide galactosyltransferase; CNS, central nervous system; CST, cerebroside sulfotransferase; EAAT2, Excitatory amino acid transporter 2; EAE, Experimental autoimmune encephalomyelitis; EGFR, epidermal growth factor receptor; EVs, extracellular vesicles; GalCer, galactosylceramide; GlcCer, glucosylceramide; GCS, glucosylceramide synthase; GPI, glycosylphosphatidylinositol; GSL, glycosphingolipids; HD, Huntington's disease; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; Htt, Huntingtin; l*d*, liquid-disordered; l*o*, liquid-ordered; LPS, lipopolysaccharide; LRP1, lipoprotein receptor-related protein 1; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MVs, microvesicles; NCAM, neural cell adhesion molecule; NF155, neurofascin 155; NGF, nerve growth factor; NgR1, Nogo receptor 1; NMDA, N-Methyl-D-Aspartate; OL, oligodendrocyte; OPC, oligodendrocyte precursor cells; PC, phosphatidylcholine; PD, Parkinson's disease; PDGFR, PDGFα receptor; PGLRs, phosphatidylglucoside-enriched lipid rafts; PLP, myelin proteolipid protein; PNS, peripheral nervous system; PrP, prion protein; PtdGlc, phosphatidylglucoside; SM, sphingomyelin; STED, stimulated emission depletion; TLR4, toll-like receptor 4.

ABSTRACT

Lipid rafts are small, dynamic membrane areas characterized by the clustering of selected membrane lipids as the result of the spontaneous separation of glycolipids, sphingolipids, and cholesterol in a liquid-ordered phase. The exact dynamics underlying phase separation of membrane lipids in the complex biological membranes are still not fully understood. Nevertheless, alterations in the membrane lipid composition affect the lateral organization of molecules belonging to lipid rafts. Neural lipid rafts are found in brain cells, including neurons, astrocytes, and microglia, and are characterized by a high enrichment of specific lipids, depending on the cell type. These lipid rafts seem to organize and determine the function of multiprotein complexes involved in several aspects of signal transduction—thus regulating the homeostasis of the brain. The progressive decline of brain performance along physiological aging is at least in part associated with alterations in the composition and structure of neural lipid rafts. In addition, neurodegenerative conditions, such as lysosomal storage disorders, multiple sclerosis, and Parkinson's, Huntington's, and Alzheimer's diseases, frequently are characterized by dysregulated lipid metabolism, which in turn affects the structure of lipid rafts. Several events underlying the pathogenesis of these diseases appear to depend on the altered composition of lipid rafts. Thus, the structure and function of lipid rafts play a central role in the pathogenesis of many common neurodegenerative diseases.

INTRODUCTION

WHAT ARE LIPID RAFTS?

When the writers discuss about lipid rafts with colleagues at dedicated meetings, we are always surprised to realize that different scientists use the term “lipid rafts” to describe biological entities that can be significantly different. In other words, “lipid rafts” does not have the same meaning for all, thus, we think that asking the question “what are lipid rafts?” is not useless. Gerrit van Meer and Kai Simons formulated the lipid rafts hypothesis in 1988 to address the question “how the molecular composition of the different cellular compartments is generated and maintained”(2). Simons and van Meer reported that the lipid composition of the apical and basolateral membranes of polarized cells from intestinal and kidney epithelia is radically different. Notably, apical membranes are strongly enriched in glycosphingolipids and cholesterol, with a GSL:phospholipid:cholesterol ratio near to 1:1:1, and a very low content in PC, a very unusual plasma membrane composition (3-8). The tight junctions, separating the apical and basolateral domains, serve as a diffusion barrier maintaining this difference (8), however to explain how it is created, van Meer and Simons hypothesized that GSL and cholesterol are sorted from glycerophospholipids along the traffic route before reaching the cell surface (convincing experimental proof for this hypothesis came only 21 years later (9)), and speculated that the ability of sphingolipids to self-associate, due to their property to form a tight network of intermolecular hydrogen bonds (10, 11), could represent the major driving force for the sorting. Later on, this concept was broadened and refined by taking into consideration the role of cholesterol (also enriched in the apical membranes of polarized epithelial cells) in stabilizing sphingolipid clusters via tight interactions with their hydrophobic hydrocarbon chains (12), and by the assumption that lipid rafts might be the result of lateral phase separation of a liquid-ordered (l*o*) phase in fluid biological membranes (13-15). In other words, the key concept underlying the lipid raft hypothesis is that some membrane lipids, due to their intrinsic features, might be responsible for the creation of lateral order within biological membranes. This concept was not particularly innovative. In the mid ‘70s, shortly

after the formulation of the fluid mosaic model by Singer and Nicholson, studies of the thermal effects on the aggregational properties of membrane lipids, in relatively simple membrane models, suggested that fluid-fluid phase separation, due to incomplete miscibility of lipids (as the consequence of molecular mismatches between different lipids), could represent a major driving force for the creation of a certain degree of lateral order within cell membranes (16-19). In 1982, Karnovsky elegantly postulated that phase separation of different membrane lipids environment could drive the "organization of the lipid components of membranes into domains" (20). On the other hand, the lipid raft hypothesis by van Meer and Simons translated this concept from biophysics to cellular biology, by speculating about the possible biological functions of lipid-driven membrane domains. The original possible biological function attributed to lipid rafts was their role in sorting different proteins along the trafficking route and in targeting these proteins to specific membrane compartments (e.g., the apical vs. basolateral membrane in polarized epithelial cells). However, for about a decade, the fortune of lipid rafts was quite limited (Figure 1). Two events mostly contributed to the sudden booming of raftology:

1) In 1992, Brown and Rose published a seminal paper reporting that apical GPI-anchored proteins from epithelial cells can be enriched in a low density, Triton X-100-insoluble fraction, enriched in GSL and depleted of typical basolateral membrane proteins (21). This experimental evidence supported the hypothesis that the association of proteins with GSL-enriched membrane domains in intracellular site might represent a mechanism for their sorting to the apical membrane. Probably even more importantly, the paper by Brown and Rose provided a working definition of lipid rafts and a putative biochemical method for their separation. Insolubility in Triton X-100 as a criterion to define lipid raft components was subsequently fiercely criticized. On the other hand, about 2,000 papers have been published using this method, and evidences obtained by alternative methods (such

as detergent solubilization using detergents other than Triton X-100, detergent-free methods for the separation of lipid rafts, and methods for the direct recognition of lipid rafts at the cell surface) highlighted the several limitations of the Triton X-100 method, however they were not able to substantially confute the main findings obtained by this method, still widely used (see (22-27) for examples of recently published papers from very heterogeneous research areas, and our recent methodological paper about the Triton X-100 method). The discussion on this topic is outside the scopes of this review, but we invite readers to refer to our previous publications for an extensive coverage (27, 28).

2) The great leap forward in the lipid raft theory was probably represented by the article entitled “Functional rafts in cell membranes” by Simons and Ikonen (29). In this paper, the authors emphasized the finding that several proteins (and lipids, even if surprisingly they mentioned about phosphoinositides and sphingomyelin, apparently neglecting two decades of research pointing out the importance of GSL as modulators of signaling pathways) involved in signal transduction were enriched in “detergent-insoluble, glycolipid-enriched complexes”, and postulated that lipid rafts might serve as “relay stations in intracellular signaling”. Within two years since the publication of this article, the number of papers per year having “lipid rafts” as keyword increased by a factor of 10 (Figure 1). Nowadays, the importance of association with lipid rafts for apical sorting of proteins, and in general of lipid rafts as a sorting machinery still remains unclear and controversial (30-37). On the other hand, lipid rafts became enormously popular and have been involved in an incredible number of different cellular functions and biological events, and dysregulation of raft-related events has been linked to a number of pathologies.

Accumulating pieces of information about the composition and possible biological functions of lipid rafts soon led researchers to realize that lipid rafts are extremely complex

entities, and that different experimental approaches are able to unveil only partial aspects of the complex their nature. Indeed, the lack of a golden standard for the study of lipid rafts led to a fierce debate questioning even the real existence of such structures, and the need of a “consensus” definition of lipid rafts vigorously emerged. In 2006 (when the number of lipid raft-related publication reached a plateau of ~400 papers, lasting for about 10 years), the Journal of Lipid Research published a consensus definition of membrane rafts, originated by the discussion within the Keystone Symposium on Lipid Rafts and Cell Function “Membrane rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipids interactions” (38). This definition had the great merit to emphasize the nature of lipid rafts as highly dynamic and heterogeneous, non-equilibrium entities, confirmed along the years by the development of different techniques allowing to directly visualize lipid rafts on the cell surface and to overcome the major limitations posed by the use of the detergent method (or other methods of isolation of lipid rafts that did not allowed to address the dynamic aspects). These techniques encompassed fluorescence recovery after photobleaching, fluorescence resonance energy transfer, single-particle tracking techniques in their different declinations, and, more recently, Stimulated Emission Depletion Microscopy (STED) (the first fluorescence microscopy technique able to break the limit imposed by the diffraction barrier, thus allowing to reach a spatial resolution at the nanometer level, together with a temporal resolution in the range of milliseconds). The interpretation of the data gathered by using these different approaches should carefully consider the great differences in terms of spatial and temporal resolution among the different techniques used. However, altogether they confirmed the main tenet of the lipid raft hypothesis, demonstrating the non-random distribution of cell surface molecules (proteins and lipids), with a high level of lateral organization with different hierarchy,

leading to the (co)existence of membrane rafts differing in their composition, size and spatial and temporal dynamics. Lipid rafts in intact cells are short-ranged structures, however their size varies between the nanometer (39-46) and the micrometer scale (47-49). They are non-equilibrium structures, with a lifespan ranging from microseconds (50-52) to milliseconds and seconds (42-48). These two features confirm that lipid rafts can undergo deep reorganization upon diverse biological stimuli.

The studies in intact cells and/or in reconstituted membranes closely approaching the complexity of the natural systems have also confirmed the importance of fluid-fluid phase separation of membrane lipids as a major (even if probably not the only) driving force in the dynamic organization of lipid rafts (hypothesized by Simons and van Meer on the basis of a huge body of experimental evidence, however deriving from studies on highly simplified membrane models). Surprisingly to us, this aspect was much underestimated in the Keystone consensus definition, probably reflecting a rather protein-centric vision of lipid rafts. Fluid phase separation has been observed in giant unilamellar vesicles formed by brush border membrane lipids (53), in vesicles derived from different cells (54-56), and in budded HIV virus membranes (57) (membrane vesicles naturally originated from cells). Cross-linking of GM1 ganglioside in plasma membrane derived from A431 cells induced lateral reorganization the membrane with the formation of micrometer-scale GM1- and cholesterol-enriched domains, able to recruit lipid-anchored proteins, and characterized by a lower translational diffusion and a higher degree of lateral order if compared to the surrounding membrane (58, 59), in reasonable agreement with what expected for a putative *lo* phase. STED microscopy confirmed that transient confinement of GPI-anchored proteins in nanoscale membrane domains in living cell membranes is dependent on sphingolipids and cholesterol (44, 45).

All considered, a reasonable definition of lipid rafts should consider as central the importance of lipid-driven lateral organization in the assembly, maintenance and dynamics

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of these structures. In this sense, sphingolipids, especially glycosphingolipids, and cholesterol are central player in lipid raft biology. We have summarized the structural features which favor the phase separation of sphingolipid- and cholesterol-enriched membrane domains ("lipid rafts") in recent review articles (60). Of course, "lipid-driven" does not exclusively implies lipid phase separation. As exemplified in the next sections, specific lipid-proteins interactions within lipid rafts definitely contribute to their biological roles. However, emphasizing the importance of lipids is crucial when discussing the role of lipid rafts in the nervous system, the tissue with the highest enrichment in sphingolipid and cholesterol, and in particular their role in neurodegenerative diseases, which, even if incredibly diverse and heterogeneous, are characterized almost invariantly by deep alterations in the homeostasis of these lipids.

LIPID RAFTS IN THE NERVOUS SYSTEM

The link between lipid rafts and the nervous system is not surprising for many reasons.

At the cellular level, the main cellular populations present in the nervous system, neurons, myelin-forming cells (oligodendrocytes in the central nervous system, Schwann cells in the peripheral nervous system) and astrocytes, are highly polarized cells with incredibly sophisticated levels of lateral organization in different membrane subcompartments.

At molecular level, in the human body, the brain is the organ with the highest content in amphipathic lipids (61-63). In particular the different plasma membrane specializations of neural cells are highly enriched in cholesterol (64, 65) and in sphingolipids, sphingomyelin and glycosphingolipids. In addition to cholesterol and sphingolipids, the classical l*o* phase-, raft-forming lipids, the nervous system is characterized by the abundancy of other lipids able to influence the organization of lipid rafts. Phosphatidylglucoside (PtdGlc) is a recently discovered, unique glycoglycerolipid (66) present in different mammalian cell types but particularly expressed in the two primary neurogenic regions of the adult brain (67). PtdGlc shares two peculiar features with GSL: the asymmetric localization in the outer leaflet of

the plasma membrane, and the ability to undergo lateral segregation with the formation of PtdGlc-enriched lipid rafts (68, 69). In addition, endocannabinoids, usually not regarded as typical lipid raft lipids, have been reported to be associated with lipid rafts in neurons (70) and microglia (71).

In the case of sphingolipids, their expression is not homogeneous in different brain areas and cellular populations. Brain gray matter and neurons are characterized by a high content in sphingomyelin and in complex polysialogangliosides (72-74). Myelin and oligodendrocytes are also enriched in sphingomyelin, on the other hand the main myelin glycolipids are galactosylceramide and in its sulfated derivatives, in particularly, 3-*O*-sulfogalactosylceramide (or sulfatide) (75, 76) (in addition, about 26% of myelin dry weight is represented by cholesterol) (62). Astrocytes and microglia are characterized by a lower sphingolipid content and by the presence of simpler glycolipid species, even if specific compositional studies for these cell types are quite rare (77, 78).

Sphingolipid expression in brain cells appears tightly regulated along development, adult life, and physiological aging. The ganglioside total amount and the molecular complexity markedly increase from the embryonic stages to the postnatal life in chicken (79), murine (80) and human brain (73), as well as *in vitro* models of differentiating neurons (79-86). Similarly, galactolipids synthesis is activated during terminal differentiation of oligodendrocytes and is maximal during the extension and wrapping of the myelin sheaths (87).

The regulated regional expression of glycolipid patterns, even if the result of a very complex metabolic and trafficking machinery (88) (89, 90) (91, 92), is mainly linked to changes in the expression and activity of the biosynthetic enzymes (glycosyltransferases). In particular, the shift in ganglioside expression observed during neuronal differentiation is obtained with the concomitant and opposite regulation of the two glycosyltransferases at the branching point in the ganglioside biosynthetic pathway (80, 90, 93).

The synthesis of complex sphingolipids is vital for the development and proper maintenance of the nervous system. Cells lacking glucosylceramide synthase (GCS) (94, 95), and thus totally deprived of glucosylceramide-based sphingolipids, do survive and grow normally. However, the global deletion of GCS in mice is embryonic lethal with total absence of cellular differentiation beyond the primitive germ layers (96), while neural cell-specific deletion of GCS is characterized by early severe neurological defects and death within 3 weeks (97). This indicates that the correct synthesis of these lipids is crucial for the complex network of cell-cell and cell-microenvironment interactions that characterize the maturation of the nervous system. Similarly, the genetic deletion of the key enzyme for the synthesis of myelin glycolipids, UDP-galactose ceramide galactosyltransferase (CGT) (98, 99), led to the production of non-functional myelin. As the result, the speed of nerve conduction in CGT-null mice is similar in myelinated and unmyelinated axons (98), and the mice show a severe hypomyelination phenotype. This is remarkable, considering that these mice indeed produce myelin sheath in amount and appearance very similar to wild type mice, due to the increased synthesis of high levels of hydroxy-fatty acid-containing GlcCer and sphingomyelin. However, the synthesis of these abnormal sphingolipids is not able to replace the function of the lacking galactolipids at the molecular level (98, 99).

The functional relevance of sphingolipids in brain cell membranes is at least in part linked to their ability to laterally compartmentalize the membrane in distinct domains. As recalled previously, sphingolipids bear at least three distinctive molecular features (28, 100-102) favoring their phase separation respect to the bulk glycerolipid membrane environment. Lateral segregation of sphingolipids is driven by the formation of a thick network of intermolecular hydrogen bonds at the water/lipid interface of the bilayer (10, 103, 104), due to presence in the ceramide backbone of functional groups acting as donors and acceptors for hydrogen bonds. The importance of this network of hydrogen bonds in stabilizing the lateral segregation of membrane lipids is highlighted by the observation that the simplest

sphingolipid, ceramide, is able to drive, by itself, the formation of lipid rafts. Ceramide has important roles as a bioactive molecule *per se*, acting on diverse targets, both at the plasma membrane level and at intracellular level. Different sphingomyelinases are present in plasma membrane or can be translocated to the plasma membrane from intracellular sites upon different stimuli. In addition, one isoform of sphingomyelin synthase is enriched in the plasma membrane. Thus, the plasma membrane ratio between ceramide and sphingomyelin can be effectively regulated at local level. In addition, ceramidases and sphingosine kinases are also associated with the plasma membrane, thus hydrolysis of sphingomyelin has been regarded by many authors mainly as a mechanism to generate bioactive ceramide and/or sphingosine 1-phosphate (105). On the other hand, ceramide itself is able to segregate within the plasma membrane forming ceramide-rich platforms, a specialized subtype of lipid rafts. Apparently ceramide rafts do represent a third type of membrane domain (in addition to the typical raft liquid-ordered phase and to the liquid-disordered non-raft membranes), characterized by a gel-like structure (106). Indeed, for some Authors the major function of ceramide generated at the plasma membrane is not that of second messenger, but rather that of modulator of membrane structure (107). The structural changes promoted by the formation of ceramide-rich rafts not only affects the segregation of membrane receptors and other signaling molecules (a classical function attributed to lipid rafts). In particular, the sphingomyelin/ceramide interconversion at the plasma membrane has potentially important consequences on the membrane organization, strongly influencing not only membrane lateral but also membrane topology and in particular curvature. Reorganization of lipid membrane domains into ceramide-rich signaling platforms has been reported to occur upon different receptor-dependent and -independent stimuli (108, 109). The dramatic change of lipid aggregational properties associated with generation of ceramide from plasma membrane amphiphilic lipids (107, 110) has been suggested to be responsible for massive rearrangements of lipid raft

organization, leading to the coalescence of pre-existing small-scale rafts into large ceramide-rich signaling platforms (108, 109), possibly coupled with changes in membrane curvature and eventual inward or outward vesiculation (100, 111). From this point of view, it is worth to note that ceramide can be generated in the plasma membrane also from glycolipids, by complete removal of their oligosaccharide chains (112).

In addition, sphingomyelin and gangliosides in neurons are rich in saturated fatty acids, whose chains are extended and ordered in the core of the lipid bilayer and can interact tightly with cholesterol (113), also present at high enrichment in brain cell membranes. The close interaction of cholesterol via its planar α-face with the ordered acyl chains of acyl-lipids, filling in the hydrophobic gaps between the acyl chains, is a key factor in the stabilization of the l*o* phase. In the case of glycosphingolipids, a further driving force for segregation is represented by the bulkiness of the hydrophilic head groups and by its potential to establish strong conformational correlations in glycolipid clusters (100).

Thus, we speculate that one of the major functional roles of sphingolipids in neural cell membranes is the formation and stabilization of lipid rafts, and that the functional importance of sphingolipids is mirrored by that of lipid rafts. However, this speculation is somewhat challenged by the incredible number of different glycosphingolipids molecular species found in the brain, resulting from the combination of the high complexity of in the hydrophilic head groups (in particular for gangliosides) (114) and to the heterogeneity in the ceramide backbone, in terms of fatty acid (115, 116) and sphingoid base composition (117, 118). In addition, we already mentioned that different brain areas and different brain cell populations are characterized by a specific glycolipid composition. This has been known for a long time (119), however we are fully appreciating the heterogeneity in the distribution of different gangliosides in the brain only in recent times, after imaging mass spectrometry was applied to the analysis of brain gangliosides. In our opinion the new findings in this sense are quite amazing. For example, imaging mass spectrometry of

gangliosides in the three distinct layers of the molecular layer of the dentate gyrus of the hippocampus revealed a striking composition difference, notably dependent on the structure of the ceramide backbone, and in particular by the presence of d18:1 or d20:1 sphingosine (120). Interestingly, differential expression of ganglioside species characterized by a different long-chain base composition has been recently reported also for other brain areas (121, 122). On the other hand, different spatial distribution depending on the hydrophilic portion (*e.g.*, different distribution of GD1a and GD1b species) was also recently reported (123). Imaging mass spectrometry applied to this field of research is still in the need of technical refinements, however it is easy to predict that it will soon unveil novel aspect in the biology of sphingolipids in the brain. Notably, this technique has been recently applied to the analysis of APP transgenic mouse brain, a model for the study of AD, unveiling deep differences in the regional alterations of ganglioside composition (124), only partially confirming previous data obtained in other AD mice models and in AD patients.

As already mentioned, phase separation of membrane sphingolipids/cholesterol is not the only lipid-dependent contribution to the lateral organization of membrane domains, driving the compartmentalization of other membrane components. The existence of direct interactions between glycosphingolipids and/or cholesterol and several membrane proteins of great functional relevance for the nervous system has been described. The binding of GM1 gangliosides to TrkA neurotrophin receptor membrane receptors has been described long time ago (125). *N*-glycosylation of the receptor is crucial for the co-localization of TrKA with GM1 within lipid rafts, suggesting that either a glycan-glycan interaction is involved, or that the conformation of the receptor able to interact with GM1 is stabilized by its glycosylation (126). More recently, molecular docking studies revealed that GM1 oligosaccharide is able to occupy a hydrophilic pocket in the TrkA-NGF complex, stabilizing it and favoring the receptor dimerization (127). Similar findings clearly suggest

that the binding of GM1 and TrkA does not simply represent a molecular mechanism for the recruitment of the protein in a specific lipid raft.

Several proteins that interact with glycosphingolipids or are preferentially associated with lipid rafts are characterized by the presence of a characteristic amino acid sequence termed the "sphingolipid binding domain". The sphingolipid-binding domains has been identified in different membrane-associated neurotransmitter receptors, such as the human serotonin1A receptor (128, 129). The sphingolipid binding motif in serotonin1A receptor has been recently characterized at the molecular level, and it has been shown to be highly conserved along evolution (130), this suggesting its functional relevance in the biology of these receptors, and possibly in other G-protein coupled receptors. Other neurotransmitter receptors, such as the human β2-adrenergic receptor (131) and the nicotinic acetylcholine receptor (132), bear distinctive cholesterol-binding domain(s), which are able to interact with different modalities with the cholesterol molecule, that, despite its apparently simple structure, is characterized by a marked asymmetry. Intriguingly, the presence of more than one cholesterol binding domain has been reported in the same membrane protein: in the transmembrane stretch of the nicotinic receptor, distinct cholesterol consensus domains, with different preference for the outer vs. the inner membrane leaflet, have been described (133). Very interestingly, glycolipid- and cholesterol-binding domains have been identified in amyloidogenic proteins relevant to major brain pathologies, including α-synuclein and β-amyloid peptide. In both proteins it is present a loop centered on a tyrosine residue (134), which is involved in their interaction with glycosphingolipids, a relevant step in the conformational transition which precedes the oligomerization and subsequent formation of insoluble fibrils. Similarly, cholesterol binding domains have been identified in the structures of α-synuclein (135), in the amyloid precursor protein (136, 137) and in β-amyloid peptide (138, 139). In some cases, the binding of a certain protein with glycosphingolipids and cholesterol is not only specific, but somewhat cooperative. For

example, α-synuclein can bind to different gangliosides at the surface of brain cells depending on the cell type (i.e., with GM3 in astrocytes, or with GM1 in neurons). In both cases, the binding with the ganglioside induces a conformational change in α-synuclein, that is permissive for a high affinity interaction with cholesterol in the plasma membrane. This, in turn, enhances α-synuclein oligomerization (140).

Clearly, we need to learn more about the cell-specific functions of different glycosphingolipids species, and of cholesterol, and about the specific lipid-protein interactions, whose repertoire is likely to widen up in the future.

On the other hand, the complexity in glycolipid distribution among different brain cell populations has important consequences also from the point of view of phase separation. Recent studies highlighted that the dynamics of lipid phase separation are much more complex than expected. Single-molecule imaging of different fluorescent GM1, GM3 and SM analogues in living cell plasma membrane has revealed that the clustering of sphingolipids around a GPI-anchored protein is the result of a series of transient events, encompassing the formation of homo- and heterodimers, small clusters and larger aggregates, where the sphingolipid molecules are in continuous and rapid exchange between the raft environment and the bulk of the plasma membrane (141-145). Thus, formation and stabilization of l*o* lipid rafts in cellular membrane is much more complex and dependent on the specific lipid composition of a given membrane that predicted on the basis of the data previously available from the study of model membranes.

Lipid rafts in neurons

Regarding neuron cell biology, membrane receptors represent the most relevant example of proteins whose functions are modulated by their association with lipid rafts. In neurons, these lipid rafts-associated receptors exhibit an extensive variety, in terms of ligand type (including endocannabinoids, neurotrophins, and several neurotransmitters), downstream

signal transduction mechanism (GPI-anchored receptors, tyrosine kinase receptors, adhesion molecules coupled with intracellular non-receptor tyrosine kinases of the Src family, and G protein-coupled receptors (72, 84, 146-155)), and dynamics of receptor association with membrane domains. For example, some receptors, upon activation, translocate from/to lipid rafts to/from a non-raft membrane region or a different population of membrane domain or other intracellular sites, thus allowing the reciprocal engagement of co-receptor molecules that do not interact in the resting state, or allowing segregation of molecules that co-cluster in the resting state. Other receptors, instead, normally reside in lipid domains while in the resting state and their activation leads to propagation of signals to other components present in these domains (156).

An example of this kind of lateral interaction is represented by the binding of GM1 to the Trk family neurotrophin receptors. GM1, who due to its neuroprotective and neurotrophic effects is being taken into consideration as therapy for different diseases characterized by neuronal damage (157-161), is able to interact with Trk neurotrophin receptor, both *in vitro* (125) and *in vivo* (162-164), substituting or enhancing neurotrophins in their actions (165). This interaction, which seems to be mediated by hydrogen bonds and ionic interactions between the oligosaccharide portion of GM1 and the extracellular moiety of Trk (127), determines receptor activation (166) and increases Trk kinase activity, NGF-dependent receptor homodimerization and autophosphorylation (159, 166-169). Moreover, the local activation of a plasma membrane-associated ganglioside sialidase, which leads to an increase in GM1 levels consequently increasing Trk activation in specific domains at the surface of unpolarized neurons, was able to locally induce actin depolymerization and to trigger axon formation (170) (Figure 2). Furthermore, responsiveness to NGF and membrane distribution of Trk are altered by anti-GM1 antibodies from patients with the most severe form of Guillain-Barré syndrome, associated with axonal pathology (171). These antibodies also inhibit NGF-induced Trk autophosphorylation (171). This example of

the role of lateral organization in regulating the fate of neurons shows how the major players, from the substrates and enzymes necessary to synthesize GM1, to the Trk receptor, to the machinery regulating actin polymerization involved in events downstream to receptor activation, all need to be associated with certain membrane domains, which are distributed asymmetrically on the neuronal surface.

Trk receptors association with GM1-rich membranes is also important for the interaction between the receptors and signal transducers such as Src family non-receptor tyrosine kinases, typically higher in neuronal lipid rafts in a cell- and stage-specific manner (72, 161, 172), and who present an activity finely regulated by gangliosides.

Lipid rafts in astrocytes

As mentioned in previous paragraphs, PtdGlc, whose saturated fatty acyl chains, C18:0 at sn-1 and C20:0 at sn-2 of the glycerol backbone, has the ability to undergo lateral segregation thus forming phosphatidylglucoside-rich lipid rafts (PGLRs) (68, 69). In astrocytes, these rafts were shown to regulate astrogliogenesis, by controlling EGFR tyrosine kinase activity during mid-embryonic to early postnatal stages of mouse brain development (173).

More conventional ganglioside-enriched lipid rafts in astrocytes are also involved in homeostasis, regulating glutamate clearance through EAAT2 modulation (174) and potassium buffering through the modulation of Kir4.1(175), and play roles in the signaling leading to ganglioside-induced autophagic astrocyte death (176). Moreover, they can modulate astrocytic inflammatory signaling. DJ-1 is a ubiquitous protein, highly expressed in both brain and peripheral tissues, that was initially described as an oncogene and whose mutations are associated with autosomal recessive forms and some sporadic cases of Parkinson disease (177). Recent evidence suggests that DJ-1 is a multifunctional protein that has potent antioxidant properties and protects neurons against oxidative stress-induced cell injury (178). Moreover, its association with lipid rafts, in fact, regulates

the inflammatory response to lipopolysaccharide (LPS) through the modulation of the LPS/TLR4 lipid raft-dependent pathway (179).

Lipid rafts in oligodendrocyte maturation, myelin formation and stabilization

Mature oligodendrocytes (OL) are the CNS myelin-forming cells. These cells go through strictly regulated differentiation steps (180) which culminate in the formation of the myelin membrane, a multilayered membrane wrapping around axons which present its own peculiar cytoarchitecture, characterized by the presence of several different functional microdomains, including caveolar domains, tetraspanin-enriched microdomains, and sphingolipid-enriched domains (181, 182). The latter are involved in several functional aspects of OL (183). For example, galactosylceramide-rich and sulfatide-enriched domains are involved in myelin stabilization (Figure 3). In fact, trans-interactions between galactosylceramide and sulfatide in opposing extracellular surfaces of the myelin sheath form specialized “glycosynapses” which increase the stability of the membrane wrapping (184-187). Moreover, oligosaccharide-mediated trans interactions between GT1b and GD1a gangliosides on the axonal surface and MAG (188-191), whose localization is regulated by galactolipid-rich domains (192), is necessary for long-term axon-myelin stability (Figure 3).

Galactosylceramide-rich and sulfatide-enriched domains also modulate the lateral distribution and co-clustering of several myelin proteins, thus regulating proliferation, survival and differentiation of oligodendrocytes (183). In early stages of myelin development, only few of the typical myelin proteins are associated with lipid rafts. By the mid-myelination stage, however, when galactosylceramide (GalCer) and sulfatide are synthesized at detectable levels, the myelin proteins PLP and MOG tend to localize in lipid rafts, and, in the final stages of myelination, MAG and MBP are also translocated into lipid rafts (192-195). Interestingly, the association of PLP to GalCer- and cholesterol-rich domains in the Golgi complex, which is a critical step sorting of components destined for

the myelin membrane, is required for correct assembly of the protein in the myelin membrane (196). Moreover, sulfatide seems to be necessary for the transport of PLP to myelin membranes via a transcytotic mechanism (197). Neurofascin155 (NF155), which associates with sulfatide and stabilizes axon-glial contacts, is also recruited into lipid rafts during the final stages of myelin development (192, 194).

Lipid rafts in microglia

Microglial cells are widely regarded as the resident immune cells of the brain, constantly scanning through the microenvironment with their long protrusions, readily sensing alterations in tissue homeostasis and integrity (198).

Lipid rafts, in these cells, are involved in several processes. For example, they play a role in lysophosphatidylcholine (LysoPC) induction of ROS production, which leads to caspase-1 activation and to the subsequent IL-1β processing. They are also involved in the internalization of α-synuclein (α-syn) through the interaction between ganglioside GM1, an unknown receptor, and the α-syn protein (199). Moreover, caveolins, membrane adaptor proteins associated with lipid rafts, have been identified as structural and metabolic regulators of microglia. In particular, it has been observed that the switch between a resting phenotype and an immunoinflammatory one is associated with a switch in the caveolin isoform expression. When cells are in the inactive state, Caveolin-1 (Cav-1) levels are low and the protein is localized in cytoplasmic vesicles and at plasma membrane level. Caveolin-3 (Cav-3) instead is highly expressed and localizes in cellular processes and perinuclear regions. Upon microglia activation, concomitantly with the changes in cell morphology, Cav-3 expression lowers, whereas Cav-1 expression increases. Cav-1 in these cells enhances mitochondrial function and acts as a negative regulator of microtubule stability, and, since lipid raft marker flotillin-1 levels increase alongside Cav-1 levels, it has been hypothesized that lipid rafts might be involved in the regulation of the morphology changes associated with the inactive-active state transition (200).

ALTERED LIPID RAFT ORGANIZATION AND NEURODEGENERATIVE DISEASES

Considering that control of lipid composition is a physiological mechanism for the modulation of lipid raft-dependent cellular functions in the nervous system, it is not so surprising that alterations of lipid metabolism, subsequently leading to abnormal lipid raft organization and functioning, are often associated with neurodegenerative diseases.

Indeed, genetic defects (lack of an enzyme activity or of an activator protein required for the enzyme activity) leading to the impairment of the sphingolipid degradation pathway at the lysosomal level cause the primary accumulation of the undegraded sphingolipid substrate in the lysosome. This is the common feature of a very heterogeneous family of lysosomal storage diseases, the sphingolipidoses. Almost all of sphingolipidoses, even if very diverse in their clinical manifestations, are characterized by severe neurological involvement and neurodegeneration. Lysosomal impairment due to the engulfment of undegraded substrate is likely the main causative factor behind the pathology; however, several papers suggest that escape of sphingolipids from the lysosome and their interaction with plasma membrane and intracellular membranes might lead to altered organization of lipid rafts, which could represent an important player in the ethiopathogenesis of sphingolipidoses. This topic has been extensively covered in a recent review (201) and will not be further discussed here.

On the other hand, the defective lysosomal metabolism of sphingolipids can in some cases lead to the generation of abnormal metabolites. The most typical case is Krabbe disease. This disease, caused by loss-of-function mutations of the enzyme β-galactocerebrosidase, is characterized by severe brain impairment, demyelination and irreversible neurological damage. The lack of β-galactocerebrosidase activity results in the elevation of its substrate, GalCer. Apparently accumulation of GalCer is per se not detrimental, however GalCer is metabolized into the lysosphingolipid galactosylsphingosine, or psychosine, and it has been hypothesized that the severe phenotype of the disease could be due to

psychosine toxicity (202). However, the molecular mechanisms underlying psychosine toxicity are poorly understood. Recently, it has been shown that psychosine accumulates in lipid rafts from brain and sciatic nerve from twitcher mice (the animal model for the infantile variant of the disease) and from human Krabbe patients, disrupting the lipid raft architecture with the consequent altered distribution of lipid raft proteins, inhibition of protein kinase C (203), and impairment of lipid raft-mediated endocytosis in neural cells (204). Remarkably, accumulation of high levels of different lysosphingolipids was detected in several othe sphingolipidoses, including Gaucher's, Fabry's and Niemann Pick diseases (recently reviewed in (205), suggesting that interference with lipid raft organization by accumulated lysosphingolipids might represent a common pathogenetic mechanism.

In this section, we will focus on several common neurodegenerative diseases whose onset is not primarily considered to be related to defects in sphingolipid metabolism. On the other hand, for different and diverse reasons, a common trait of these diseases are alterations in sphingolipid and cholesterol homeostasis, and convincing evidence indicate that the resulting anomalous composition/organization of lipid rafts is an important causative element of the neurodegenerative manifestations.

However, before analyzing the changes of lipid raft composition associated with several neurodegenerative diseases, it is worth to recall that a variety of alterations of brain lipid composition have been correlated with the process of physiological aging. Along aging, brain faces a progressive overall reduction of its total lipid content, alterations of polyunsaturated fatty acid content and profile, decreased ganglioside content, and altered sphingoid base composition of SLs (for review see (206)). It is difficult to prove a causative relationship between these numerous and complex lipid composition changes and the gradual decline of physiological performance that occurs during brain aging. However, there is some recent evidence that these changes have major effects on the

physicochemical properties of lipid rafts (*e.g.*, local membrane microviscosity). which seems to associate with the decline of physiological performance of the aging brain. In other words, lipid rafts seem to undergo a natural aging process. As mentioned above, gangliosides in particular have been reported to decrease along aging in human and mouse brain. The trends of variations are quite complex, and differ in different brain areas and depending from the age range considered (73, 74, 207-209). The most pronounced changes in ganglioside composition associated with aging were an increase in the simpler gangliosides species, paralleled by a reduction of the complex gangliosides of the a-pathway (GD1a and GT1a) (74, 209). The progressive loss of brain gangliosides observed along aging (73, 74, 207-209) has been hypothetically associated with reduced neuronal and synaptic plasticity, which are in many aspects controlled by lipid rafts. On the other hand, we reported that ceramide is enriched in lipid rafts in aging cultured neurons (210). Importance of lipid rafts with abnormal organization, for example, has been suggested in Huntington's disease, Parkinson's disease and Alzheimer's disease and examples of this will be described in the following paragraphs.

In some cases the abnormal composition of lipid rafts has specific consequences for a given neurodegenerative disease. On the other hand, there are common raft-dependent mechanisms potentially contributing to the onset of different diseases. For example, as described before and discussed in specific in the next sections, association with lipid rafts with abnormal composition seems to be an important player in the amyloidogenic processing and in the aggregation of Aβ peptide and α-synuclein.

More recently, lipid rafts have also emerged as potential modulators of the genesis and functions of extracellular vesicles (EVs). EVs are membrane vesicles released by cells, characterized by highly heterogeneous size, structure, and molecular content (211). EVs are produced by all cells of the nervous tissue and have been found to play physiological

functions as well as pathophysiologic roles in inflammatory and degenerative diseases (212-214). In eukaryotic cells, EVs can derive from the plasma membrane (called ectosomes or microvesicles (MVs)) as well as from the multivesicular bodies (called exosomes) and it has been shown that exert their function as mediators of cell-to-cell communication (215-218). In this respect, the hypothesis that modulation of lipid raft composition and lateral heterogeneity might serve as a determinant for inclusion/exclusion of membrane lipids and proteins into MVs, and that these MVs could originate from specific membrane microdomain is becoming more and more popular (219).

Intriguingly, exosomes contain and diffuse different pathogenic proteins such as α-synuclein and amyloid precursor protein (220-222). In 2012 Russo et al. published a paper demonstrating that exosomes, secreted by both neurons and activated microglia, embitter neuronal dysfunction and accelerate PD progression because participate in spreading around α-synuclein and increasing neuroinflammation (223).

It is very important to underline that, not only the protein cargo of EVs, but also the sphingolipids in the EV membrane can have a fundamental role in neurogenerative disease. There are several evidence in the literature demonstrating an important role of EV sphingolipids in neurodegenerative pathological conditions in which EVs are involved. In particular Yuyama et al. (224) demonstrated that glycosphingolipids present at the exosome surface are involved in the pathogenic aggregation of the Aβ peptide. Exosomal glycolipids forming clusters (rafts?) able to bind to Aβ peptide, and these complexes behave as templates for further Aβ aggregation. Yuyama et al., on the basis of these results together with the results published by Yanagisawa et al. (225) demonstrating that GM1 associates with Aβ peptide in the brain of AD patients, suggested that in exosomes there are specific areas enriched of glycosphingolipids that bind to Aβ peptide and induce its aggregation.

Other evidence demonstrates that exosomes are highly enriched in cholesterol and sphingomyelin that, on their turn, stimulate Aβ peptide assembly promoting the lateral packing of gangliosides on membranes (224, 226, 227).

Thus, the organization of different lipids at the surface of exosomes and other EVs might represent a still poorly investigated aspect of the involvement of lipid rafts in different neurodegenerative diseases.

Lipid rafts in Huntington's Disease

Huntington's disease (HD) is a monogenic, progressive neurodegenerative disorder with an autosomal dominant pattern of inheritance. Its cause is a mutation in the huntingtin gene resulting in a polyglutamine expansion in the *N*-terminus of huntingtin (Htt) (228, 229), a scaffold protein involved in transcriptional control of neural gens, autophagy, and vesicular traffic (230). Ganglioside synthesis and expression of the glycotransferases involved in this process are altered both in cellular and animal models of HD (231-233) and in the striatum of HD human brains (234). GM1, in particular, decreases markedly (38% reduction vs wild type) and this reduction correlates with an increased susceptibility to neuronal death. GM1 administration is able to restore normal survival in HD cells in vitro, via activation of the PI3K/Akt pathway and huntingtin phosphorylation (233). Interestingly, sphingosine-1-phosphate (S1P) metabolism is also altered in several models of HD, and treatment with fingolimod is able to restore GM1 normal levels in HD mice (233, 235).

Cholesterol levels are also altered in HD. Cells expressing mutated Htt have an increased content of cholesterol, in particular in lipid rafts, together with an enrichment of the GluN2b subunit of NMDA (N-Methyl-D-Aspartate) ionotropic glutamate receptors in these domains (236). Moreover the expression of cholesterol hydroxylase enzyme CYP46A1, which is downregulated in HD, is neuroprotective against mHtt induced toxicity both in in vitro and

in vivo models of HD (237). This enzyme is protective against NMDAR-mediated excitotoxicity in HD models however, while CYP46A1 is able to reduce cholesterol content in lipid rafts in wild type neurons, its overexpression in HD neurons was not able to restore normal cholesterol levels in these domains (238).

It has been proposed that the alteration in cholesterol metabolism could be underlying the myelin deficits in HD (239). White matter abnormalities appear early in the course of the disease and worsen with age (240). Moreover, mice expressing reduced levels of Htt show alterations in OPC maturation and white matter tract impairments during development(241), and OPCs isolated from neonatal HD mouse brains and derivative oligodendrocytes exhibit deficits in the levels of myelin-related genes ((242). The levels the two major glycosphingolipids in myelin, galactosylceramide (GalCer) and sulfatide, are also decreased in a mouse model of HD (232).

Lipid rafts in Parkinson's disease

Cholesterol balance in the brain and cholesterol serum levels are altered in of Parkinson's disease (PD) patients (243, 244). Several proteins whose mutations have been causally correlated with PD, including parkin, PINK1, α-synuclein, and DJ-1, have been detected in lipid rafts in the brain, neurons, and astrocytes (179, 245-247). In fact, parkin regulates expression of caveolin 1, altering lipid rafts and the cell to cell transmission of α-synuclein (248).

α-synuclein is involved in lipid trafficking into the cell. It binds fatty acids and acts as a transport facilitator between cytosol and membrane compartments and modulates the uptake of fatty acids in the neuronal membrane (249). Moreover, α-synuclein can modulate lipid metabolism by reducing the hydrolysis of lipid droplets (250) and plays a role in lipid membrane homeostasis, via inhibition of phospholipase D1 and D2 (251), and organization of membrane components (252).

The expression of several ganglioside biosynthetic genes is reduced in PD, consistently with the reduction in the levels of GM1, GD1a, GD1b and GT1b observed in the substantia nigra of PD brains (253). Association of α-synuclein with lipid rafts affects both the trafficking and the three-dimensional structure of the protein, and α-synuclein has been shown to interact with gangliosides and cholesterol (199, 246). Moreover, binding of α-synuclein to GM1 inhibits fibril formation and binding and specificity of GM1 are enhanced by *N*-acetylation of α-synuclein (246, 254). Furthermore, inhibition of GD3 synthase has been shown to have neuroprotective properties in the MPTP mouse model of PD (255).

Major lipid alterations in lipid composition, including a marked decrease in GalCer and sulfatide, have also been observed also in lipid rafts purified from the frontal cortex of PD patients (256).

Lipid rafts in Alzheimer's disease

The amyloid precursor protein (APP), a transmembrane protein enriched in neurons, is not a raft protein per se, however, a significant amount of APP tends to localize in lipid rafts after palmitoylation (257). The Src kinase family member Fyn and Dab1 are essential for the targeting of APP to lipid rafts, which is essential for both its physiological function and its pathological processing (258).

APP cleavage is usually considered to be modulated within lipid raft microenviroment (259) and these microdomains contain APP-derived proteolytic fragments and enzymes involved in APP amyloidogenic processing. Moreover, Aβ production is preferentially localized within lipid rafts (260).

Cleavage of APP by γ-secretase leads to the formation of Aβ and to the release of the APP intracellular C terminus domain (AICD), both able to affect cellular lipid composition (261). In particular, AICD released from APP can affect lipid raft composition through the regulation of plasmalogen synthesis (mediated by the regulation of alkyl dihydroxyacetone

phosphate synthase expression (262)) and of SL synthesis (mediated by the regulation of serine-palmitoyltransferase expression (263)).

Both cholesterol and sphingolipid metabolism are altered in Alzheimer's disease (AD). Evidence on the role of cholesterol in AD is controversial. While there is a clear correlation between mutations of ApoE and the genetic risk of developing AD, opposite findings have been reported on cholesterol levels, precursors and metabolic enzymes in the brain of AD patients, and various studies have tried to correlate levels of the circulating lipids or lipid lowering treatments to AD risk however results are controversial and results non-conclusive (264, 265). In fact, AICD downregulates low-density lipoprotein receptor-related protein 1 (LRP1), also known as ApoE receptor, thus regulating both ApoE and the cholesterol levels in the brain (266). Moreover, APP knockout increases both ApoE activity and cholesterol levels (266). On the other hand, increase of toxic Aβ oligomers, rather than non-toxic monomer, leads to an increased synaptic cholesterol concentration, accompanied by an increased activation of a cholesterol ester hydrolase (CEH), with consequent decrease in cholesterol ester concentrations (267-270).

If we consider sphingolipid metabolism, there are several alterations in AD. Sulfatide and sphingomyelin are decreased in AD post-mortem brains of individuals with pre-clinical or early stage disease (271). Moreover sphingomyelin (SM) plays a role in APP regulation. In fact, sphingomyelinases activation, with consequent SM depletion, promotes abnormal APP processing and cellular trafficking, while Aβ accumulation activates sphingomyelinase and mediates the cleavage of SM (272). Furthermore, ganglioside metabolism is altered in both animal models and human AD. The pattern of alterations of these lipids however is highly complex and depends on factors such as age of onset and type of mutation. In fact, decreased ganglioside levels with altered ratios between a-series and b-series structures and elevated levels of simpler gangliosides have been reported in several AD brain

regions (119, 273-279). Expressions of genes involved in the sphingolipid metabolism, for example GD synthase, were also found altered in brains of AD patients (280). GD3 increases APP cleavage by γ-secretase, leading to the formation of Aβ and AICD which, through two different mechanisms, work synergistically to block the production of GD3. Aβ binds GM3, rendering it unavailable for GD3 synthase while increasing the overall ratio of a-series:b-series ganglioside, while AICD, interacting with the adaptor protein F365, decreases GD3 synthase at transcriptional level (273).

GM1 and GM2 were found to be higher in lipid rafts from the frontal and temporal cortices of AD patients (281). GM1, in particular, while it has a neuroprotective effect in vivo (282-284), has been shown to increase Aβ generation and aggregation in vitro (285).

Moreover, GM1 regulates Aβ membrane binding and its associated structural changes, oligomerization and fibrillation in a cholesterol-stimulated manner (286-289). The multiple roles of lipid rafts underlying AD are summarized in Figure 4.

Lipid rafts and multiple sclerosis

Multiple sclerosis is not primarily a neurodegenerative disease; however, neurodegeneration is the unavoidable long-term consequence of myelin loss in MS patients.

Cholesterol is one of the main components of the myelin sheath. Most of the transcripts of genes involved in cholesterol biosynthesis are downregulated both in MS animal models and in MS human brains (290-292). Moreover, it has been hypothesized that this downregulation could be inhibiting the fast and efficient remyelination in MS (291). In fact, an overall downregulation of the genes of the cholesterol biosynthetic pathway has been reported in MS in humans, suggesting a possible role of cholesterol in demyelination and remyelination (293). Cholesterol ester, unlike in AD, is accumulated in MS lesions (294). On the other hand, inhibition of HMG-CoA reductase leads to a reduction both in demyelination and in inflammation in the EAE model (295).

ApoA-I may also play a role in MS. In fact, a negative correlation between ApoA-I levels and the worsening of the symptoms in MS has been reported. MS patients in an advanced state of the disease have lower plasma levels of ApoA-I compared to subjects with stable relapsing remitting disease and healthy age-matched controls (296). Fingolimod has been shown to reduce cholesterol toxicity in human macrophages in vitro, through increase of ATP binding cassette transporter A1 (ABCA1) levels and, consequently, of endosomal cholesterol efflux to ApoA-I (297). Statins are also able to increase ApoA-I levels however their use for MS therapy have delivered conflicting results (298-302).

LINGO1, a functional component of the Nogo receptor signalling complex that participates in a NgR1-p75/TROY-LINGO1 multisubunit complex, negatively regulates oligodendrocyte differentiation, while the ErbB2 receptor positively regulates it (303-305). Due to their role in OL differentiation, deregulation of LINGO1 and ErbB2 could also be involved in MS and their reciprocal regulation is tied to lipid rafts. In fact, LINGO1 inhibits ErbB2 translocation to lipid rafts and reduced LINGO1 activity correlates with an increased ErbB2 phosphorylation and an increased oligodendrocyte differentiation *in vitro* and *in vivo* (305).

CONCLUDING REMARKS

Lipids in the brain are major components, involved in processes such as neurogenesis, signal transduction, neuronal communication, membrane compartmentalization and modulation of gene expression. Due to their structural and physiological roles, alterations in lipid metabolism could reflect an aberrant brain function. It is the case of neurodegenerative pathologies such as AD, PD and HD, where alterations of membrane lipid composition and lipid homeostasis have been reported. These alterations in lipid composition subsequently determine alterations in lipid raft composition, thus affecting their physicochemical properties and the function of raft-associated proteins in neurodegenerative diseases. The development of new technologies able, for example, to

circumvent the optical diffraction limit is proving crucial to shed light on lipid rafts composition and dynamics. In fact, during the past years new techniques that allow not only direct visualization, but also quantitative characterization of nanoscopic structures have emerged (306). Moreover, since the field of super-resolution optical methods is active and constantly improving, it is likely that in the next years researches will be able to analyse the dynamics of objects with molecular scale precision at subsecond time scale. Furthermore, combination of different techniques such as electron microscopy and super-resolution fluorescence microscopy could provide detailed information on the organization of nanoscale molecular structures. Considering all this, the developing of technologies allowing to directly visualize dynamics of molecules within the rafts and to provide quantitative information thus leading to a more precise determination of lipid rafts composition and characterization of their effects on protein/protein and lipid/protein associations could allow to define new potential therapeutic targets for these diseases.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

FIGURES

Figure 1. Results of PubMed search using the keywords ‘lipid rafts’, from 1990 to 2019.

Figure 2. Schematic model for GM1/TrkA-dependent axon specification in neurons. Local activation of sialidase Neu3 in specific membrane domains, with consequent increase in GM1 levels, increases local recruitment of activated NGF receptor TrkA (pTrkA). Effectors PI3K and Rac1 are consequently recruited, ultimately leading to rapid initial polarized

outgrowth, thus triggering axon formation. Reproduced with modifications from (156), with permission. © Elsevier

Figure 3. Glycolipid-enriched membrane domains in myelin. Glycolipid-glycolipid and glycolipid-protein interactions have various roles in myelin formation, maintenance and functioning but also in axon-myelin stability and communication. On one hand, GD1a and GT1b, enriched in axonal lipid rafts, interact with MAG which in turn interacts with Nogo-R1 (NgR1) who, through interaction with p75/TROY and LINGO-1, activates RhoA leading to axon outgrowth inhibition. On the other hand, GalCer and sulfatide on opposing membrane surfaces in myelin sheaths interact with other via a trans interaction forming a "glycosynapse" which results in clustering of membrane domains and loss of cytoskeleton integrity, thus leading to the formation of mature myelin. Reproduced from (156), with permission. © Elsevier

Figure 4. Roles of lipid rafts in AD. Targeting of APP to lipid rafts with an altered composition can disrupt normal APP-dependent signal transduction and promote APP proteolytic processing via the actions of β- and γ-secretases. Moreover, AICD, released intracellularly, can affect the lipid composition of lipid rafts, while membrane-bound Aβ in lipid rafts, following its interaction with GM1, triggers the formation of insoluble amyloid fibrils and the release of toxic soluble Aβ aggregates. Interestingly, these aggregates need to interact with lipid raft-associated PrP to exert their negative effects on neurons. Reproduced from (307), with permission. © Wiley

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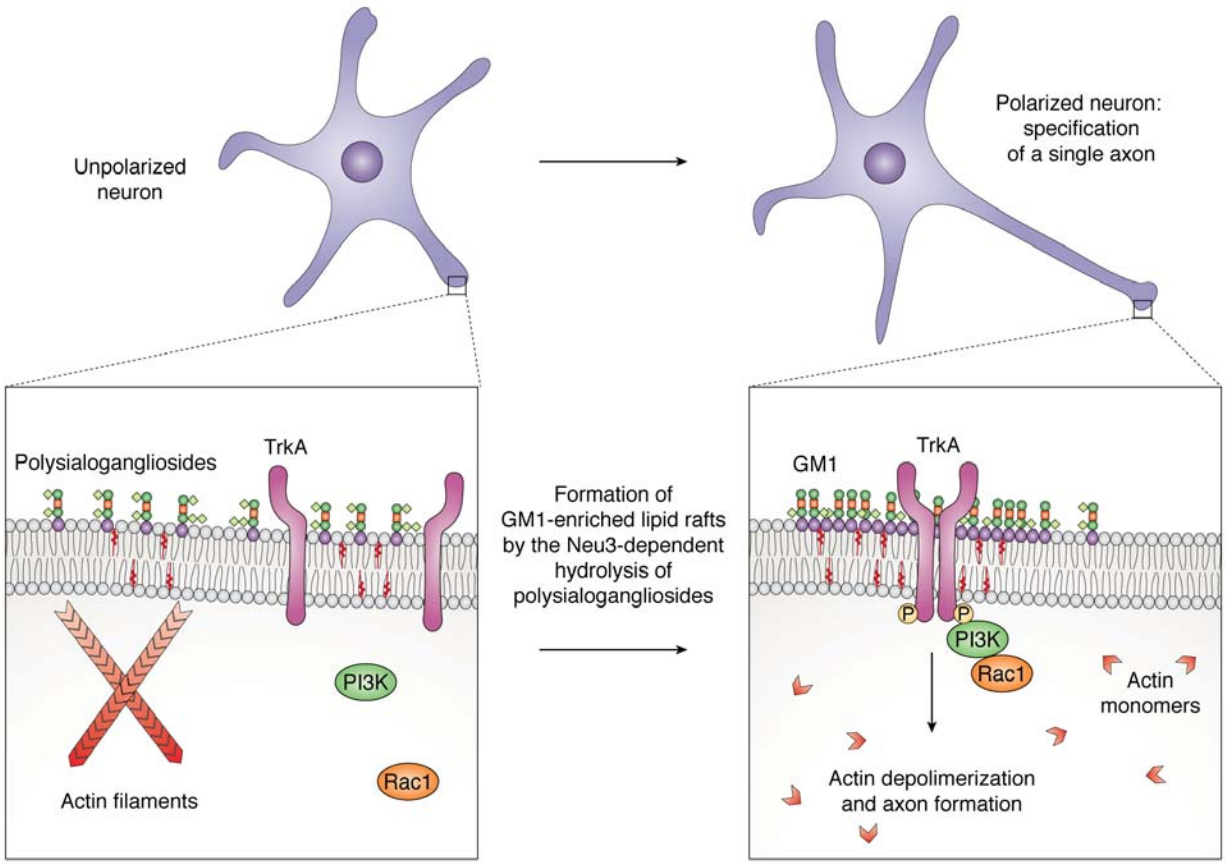
Figure 2



igure 3

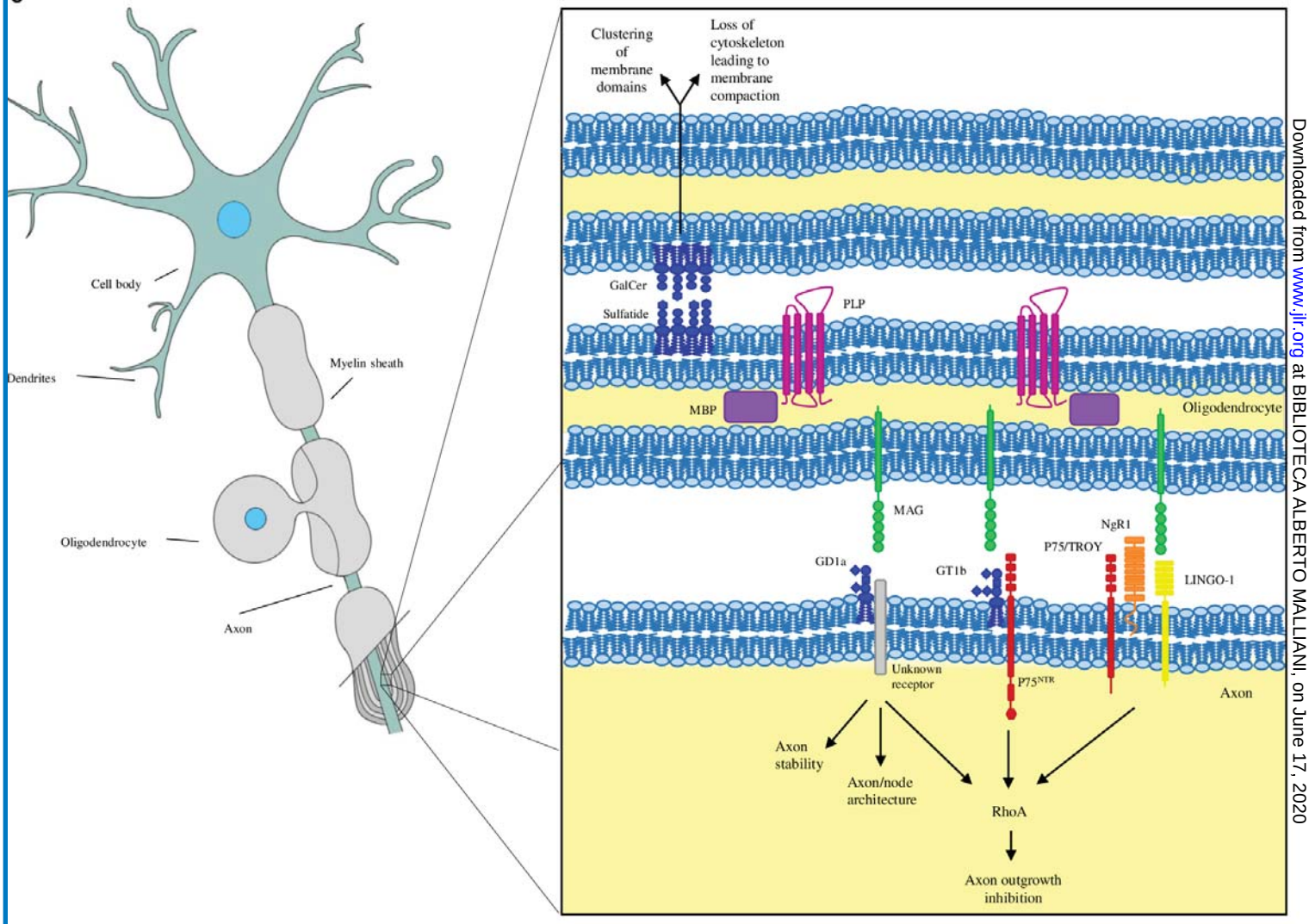
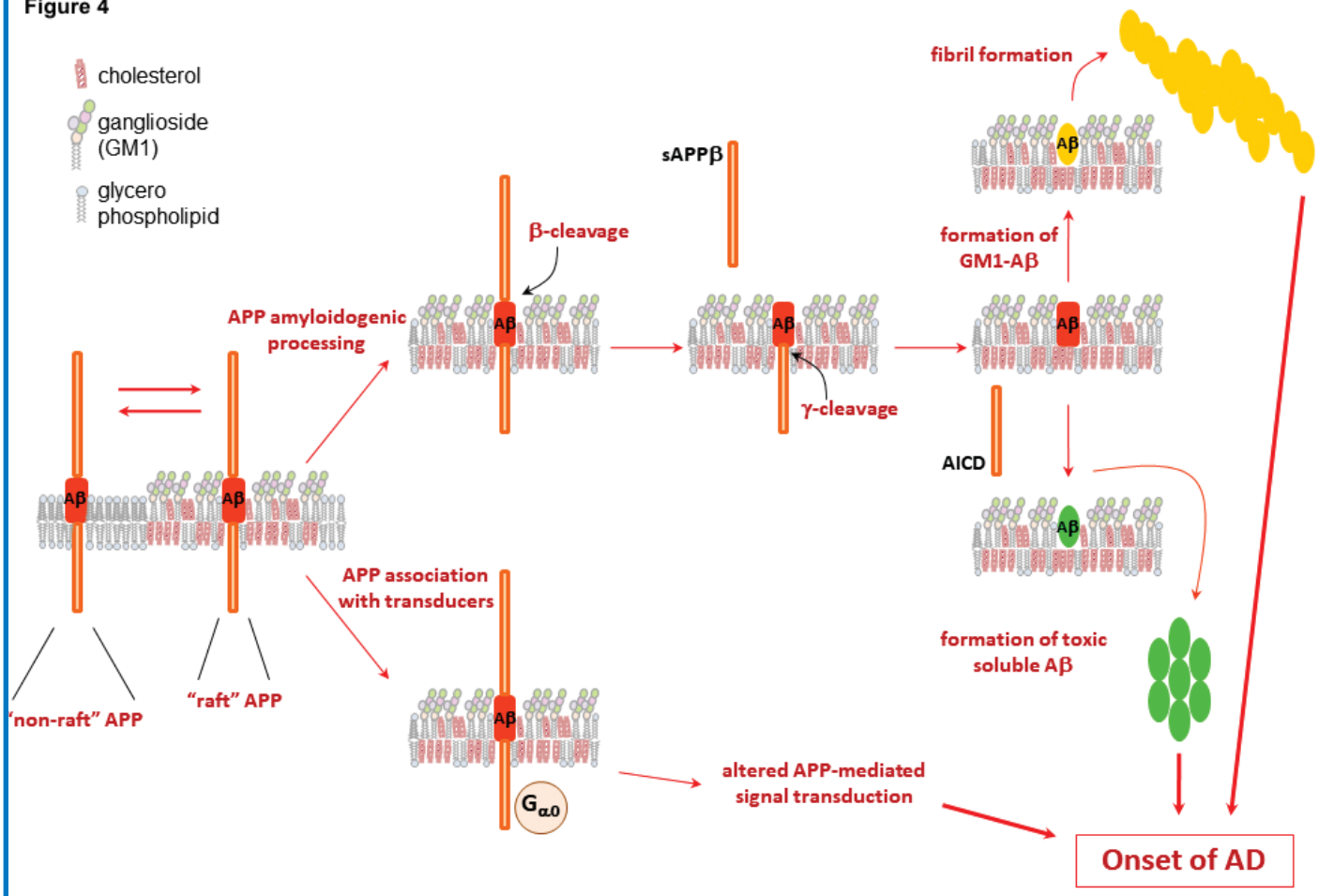


Figure 4



Graphical abstract