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Membrane Lipid Signaling in Aging and Age-Related Disease

Mark P. Mattson
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Membrane Lipid Signaling in Aging and Age-Related Disease

PREFACE

The lipids that comprise membranes of cells, be they phospholipids, sphingolipids, glycolipids or even cholesterol, serve many functions beyond their role in the structural organization of the membranes. In response to stimulation by various agonists, and environmental insults such as oxidative stress, several different membrane lipids are cleaved either enzymatically or non-enzymatically. Products are thereby released that act as important signals mediating physiological responses to the stimuli. Among the most intensively studied signaling pathways is the inositol phospholipid signaling, which leads to the production of IP3 and diacylglycerol with consequent calcium release and protein kinase activation. Another example of a prominent signaling pathway involves sphingomyelin hydrolysis resulting in the production of ceramide, which acts as an important signal in both physiological and pathophysiological settings. Recently there have been a flurry of studies characterizing so called lipid rafts, which are complex microdomains in membranes in which sphingolipids and cholesterol are concentrated, together with various receptors and transducing proteins for an array of growth factor, cytokine and neurotransmitter signals. In this issue of *Advances in Cell Aging and Gerontology* entitled **Membrane Lipid Signaling in Aging and Age-Related Disease** experts in the fields of lipid signaling and aging provide timely reviews of specific aspects of membrane lipid signaling from the perspectives of aging and diseases of aging including cardiovascular disease, cancers and neurodegenerative disorders. It is becoming quite clear that alterations in sphingolipid, inositol phospholipid and cholesterol metabolism occur in a variety of tissues throughout the body during aging, and that specific abnormalities in these signaling pathways play roles in many different age-related diseases.

The book begins with a chapter by Chris Fielding which provides an overview of signaling in the microdomains called lipid rafts and caveolae. The localization of these membrane microdomains in strategic positions within cells provides spatial control over signaling. This spatial control is particularly critical in structurally complex cells such as neurons. Kathleen Montine and colleagues then provide a review of how oxidative stress effects membranes and how the generation of lipid peroxidation products plays a role in aging and diseases of aging. Oxidative stress is considered an important factor in aging and membrane lipid peroxidation has been implicated in a variety of disorders including atherosclerosis, cancers and Alzheimer's disease. Studies of invertebrate systems where genetic manipulations can be readily performed to identify genes of interest have made major contributions to our understanding of the genes that control lifespan. Cathy Wolkow reviews studies that have identified lipid related signaling pathways that appear to play a major role in controlling lifespan. In particular, inositol phosphate signaling pathways activated by insulin and insulin-related signals appears to play an important role in coupling environmental stress signals to energy metabolism and thereby may regulate cellular aging rate.

Cancers continue to be a major killer in the United States and other industrialized countries. Ruvolo et al. review the evidence that altered sphingolipid metabolism and ceramide production play a role in the pathogenesis of cancers by altering cell cycle and cell death pathways. Altered sphingolipid metabolism also plays a role in the pathogenesis of cardiovascular disease. Atherosclerosis is a complex process involving damage to vascular endothelial cells and inflammatory processes. Sphingolipid signaling appears to play important roles in several different steps in the process of atherosclerosis, and studies of sphingolipid signaling in atherosclerosis are revealing novel targets for therapeutic intervention. In the nervous system sphingomyelin signaling plays important roles in regulating development and plasticity of neuronal circuits. Mattson and Cutler describe the roles of sphingomyelin and ceramide signaling in brain aging and the pathogenesis of neurodegenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis. It appears that increased oxidative stress in these disorders leads to excess production of ceramides and cholesterol esters which can trigger the degeneration and death of neurons.

Arachidonic acid is a product of phospholipase A_2 activation that plays important roles in inflammatory responses and also has a broad array of functions in cells in many different organ systems including the nervous system. Hari Manev and Tolga Uz describe eicosanoid pathway changes during aging of the nervous system with a focus on the regulation of cyclooxygenases and lipoxygenases. It appears that inflammation-like processes occur during brain aging and in neurodegenerative disorders, and a better understanding of arachidonic acid metabolism is therefore likely to lead to new approaches for preventing and treating neurodegenerative disorders as well as other diseases. Alterations in cholesterol metabolism are well known to play important roles in the pathogenesis of cardiovascular disease. However, recent findings have demonstrated important roles of cholesterol metabolism in modulating various signal transduction pathways and suggest that perturbed cholesterol metabolism plays roles in many different age-related diseases. John Incardona describes how studies of inherited disorders of cholesterol and sphingolipid metabolism have provided insight into the functions of lipid signaling in human disease. He describes Niemann–Pick C disease and Smith–Lemli–Opitz syndrome as two examples that have increased our understanding of the various roles of membrane cholesterol and lipid rafts in cellular biology and disease. Suzana Petanceska et al. review the emerging evidence that alterations in cholesterol metabolism play a role in the pathogenesis of Alzheimer's disease. Interesting relationships between cholesterol metabolism and the production of amyloid beta peptide, the main component of plaques in the brains of Alzheimer's disease patients, have emerged from epidemiological and experimental studies in animal models of Alzheimer's disease.

Eva Hurt-Camejo and colleagues describe reactions of phosholipases A_2 in cellular and metabolism with a focus on a role of these enzymes in the pathogenesis of cardiovascular disease. These enzymes exist both within cells and outside cells and have actions in both locations that may contribute to alterations in lipoprotein and lipid metabolism and the activation of inflammatory pathways

in the process of atherosclerosis. Finally, Nicolas Bazan provides a concise review of the role of docosahexaenoic acid in aging and age-related disease with a specific focus on glaucoma, a major cause of blindness in elderly individuals. The production and metabolism of docosahexaenoic acid display unique features. Of particular interest is the enrichment of docosahexaenoic acid in excitable membranes of the retina and brain. Several messengers are derived from docosahexaenoic acid including neuroprostanes and hepoxillins; these lipid mediators may have important roles in aging and age-related disease.

Collectively the information contained in the chapters of this volume of *Advances in Cell Aging and Gerontology* provide the reader with a clear picture of the very important roles of membrane lipid signaling in the regulation of various physiological processes throughout the body. It is quite clear that alterations in several lipid-signaling pathways occur during aging and in age-related diseases. At least in some cases these alterations may be early and pivotal events in disease processes as suggested by recent studies documenting beneficial effects of interventions that target lipid signaling. We expect that this book will be a valuable resource for investigators in fields of aging and age-related disease and that the information contained within these pages will foster new experiments aimed at unraveling the roles of lipid signaling in cellular physiology and disease.

MARK P. MATTSON, PhD

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Overview: Spatial control of signal transduction by caveolae and lipid rafts

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Contents

1. Introduction

The cell surface is heterogenous, consisting of microdomains with varying lipid and protein composition. Well-recognized microdomains include coated pits (sites of the internalization of many macromolecules) and the microvilli that identify the absorptive surface of many epithelial cells. An additional class (DRMs: detergent-resistant microdomains) has been distinguished by its insolubility in neutral detergents. This fraction is rich in free cholesterol (FC) and sphingolipids, signaling proteins, GPI-anchored proteins and FC-binding caveolin proteins. More recent research has shown that this fraction represents a mixture of two distinct species of microdomains, lipid rafts and caveolae (Fielding and Fielding, 2002).

Lipid rafts are planar microdomains, typically 50–350 nM in diameter, rich in GPI-anchored proteins but deficient in caveolins, the structural proteins of caveolae. Caveolae are invaginated domains, whose openings at the cell surface are typically 60–120 nm in diameter. These microdomains, rich in caveolin, are deficient in GPI-anchored proteins (Iwabuchi et al., 1998; Abrami et al., 2001; Sowa et al., 2001).

Caveolae and lipid rafts probably coexist in most cells, though the relative number of each varies widely in different cells. Caveolae are enriched in terminally

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differentiated cells such as adipocytes, vascular smooth muscle and endothelial cells. Their numbers are very low in blood leucocytes, which are rich in lipid rafts. As a result, leucocytes have been used for much of the published characterization of lipid rafts (Matko et al., 2002). Under basal conditions some cancer and transformed cell lines lack caveolae and caveolin entirely (Koleske et al., 1995; Lee et al., 1998). However, even these cells may express large numbers of caveolae under some experimental conditions. For example, human MCF-7 cancer cells express caveolin and caveolae concomitant with the upregulation of the p-glycoprotein transporter following incubation with adriamycin (Lavie et al., 1998).

The expression of caveolin is necessary but not sufficient for the development of morphological caveolae. In addition to caveolae, caveolin is also present in several intracellular pools, including the *trans*-Golgi network, a chaperone complex carrying newly synthesized FC, and recycling endosomes (Fielding and Fielding, 1996; Uittenbogaard et al., 1998; Gagescu et al., 2000). While the presence of caveolin in cell and cell membrane fractions does not necessarily imply the presence of cell surface caveolae, in most primary cells the plasma membrane contains the bulk of caveolin. In transformed cells, what caveolin is present may be largely in the form of intracellular vesicles (Sowa et al., 2001).

Caveolin binds FC via a central domain which also interacts with a "scaffold" sequence present on many transmembrane signal kinases, as well as a number of signal intermediate proteins, such as *ras*, and protein kinases A and C (Smart et al., 1999). Protein–protein association is in many cases dependent on or facilitated by post-translational lipid modifications (*N*-palmitoylation, *N*-myristoylation) (Sowa et al., 1999). As discussed more fully below, transport of caveolin to the cell surface and caveolar assembly may depend on these modifications.

Signaling proteins and GPI-anchored proteins bound to lipid rafts usually lack a recognizable scaffold-binding site. Their association with rafts depends both on *N*-acylation and on the presence of amino acid sequences recognize lipids (Fielding and Fielding, 2002). FC levels in lipid rafts are reported to be lower than those in caveolae (Iwabuchi et al., 1998). Ganglioside GM1, once considered a marker for caveolae, is present in both caveolae and lipid rafts. The same distribution may hold for other lipids involved in signal transduction, such as ceramide, phosphatidic acid and diglyceride. Enzymes which generate these lipids, including sphingomyelinase and phospholipase, are present in caveolae and lipid rafts. This suggests that lipid signals may be generated in caveolae. Lipid signals may in some cases extend the effect of protein-mediated signal transduction (Igarashi et al., 1999).

There is considerable difference in the lifetime of lipid rafts and caveolae at the cell surface. Lipid rafts are labile, with a measured half-life of only a few minutes, similar to the time required for signal transduction (Sheets et al., 1997; Pralle et al., 2000). They may be stabilized by the presence of FC, GPI-anchored and other proteins. In contrast, caveolae (with the possible exception of those in endothelial cells) remain at the cell surface over periods of many hours (Thomsen et al., 2002). However, while caveolae are stable, their association with signaling molecules is dynamic (Liu et al., 2000; Fielding et al., 2002).

Although caveolae and lipid rafts have a similar lipid composition, there is little to suggest that they are interconvertible. They appear to play different roles in the cell. However, crosstalk between caveolae and lipid rafts has been identified (Abrami et al., 2001). Overexpression of caveolin and caveolae was associated with a decrease in GPI-anchored proteins in rafts. Loss of GPI-anchored proteins was coupled with an upregulation of caveolin and caveolae. While under basal conditions individual signaling proteins may be associated mainly with lipid rafts, or mainly with caveolae, a change in the proportions of rafts and caveolae can lead to a redistribution of these proteins, though modification of their kinetic properties (Sowa et al., 2001; Vainio et al., 2002).

2. Functions of rafts and caveolae – signaling and FC homeostasis

These roles have been studied in detail for both classes of microdomains. Signaling via both lipid rafts and caveolae is FC-dependent. Unlike the situation with caveolae, rafts may play only a minor or negligible role in whole cell FC homeostasis. Additional roles for caveolae have been postulated in the uptake of folate via its receptor (FR), a GPI-anchored protein; in the uptake and release of certain bacteria and viruses; and (in endothelial cells) in the transcytosis of albumin. The first two of these functions now seem more likely to depend on lipid rafts, not caveolae. Caveola-mediated transcytosis has not been described except in endothelial cells. The present overview focuses on the signaling and FC-dependent roles of caveolae and lipid rafts, both because these appear to be the best established, and because recent evidence indicates them to be linked.

Of signaling pathways involving caveolae, that mediated by the platelet-derived growth factor receptor (PDGF-R) is among the best characterized. In primary cells (vascular smooth muscle cells, fibroblasts) PDGF-R is present mainly in caveolae (Liu et al., 2000; Fielding et al., 2002). Following PDGF binding, and dimerization and autophosphorylation of PDGF-R in a complex that includes the exchange factor *sos* and the small GTPase *h-ras*, independent phosphorylation cascades are mediated via the p38, PI3 kinase, ERK1/2 and other pathways. The primary sequences of PDGF-R and *ras* and caveolin itself include caveolin-binding "scaffold" sequences (Smart et al., 1999). Caveolin is also irreversibly *N*-palmitoylated (Parat and Fox, 2001). *H-ras* is both myristoylated and palmitoylated and these modifications play a key role in its association with caveolae (Prior et al., 2001). In continuous cell lines, where the expression of caveolae is often much reduced, an association of PDGF-R with lipid rafts instead of caveolae has been reported (Matveev and Smart, 2002). While initial studies suggested that caveola-associated signaling proteins represented an inactive reservoir (Smart et al., 1999), subsequent findings were made of active signaling from caveolae (Gustavsson et al., 1999; Zhu et al., 2000). However, caveolar FC content was a major regulatory factor in all cases.

Caveolae are the terminus within the plasma membrane for newly synthesized FC as well as recycling lipoprotein-derived FC. In fibroblasts and vascular smooth

muscle cells, FC loading was associated with both induction of caveolin synthesis as well as increased expression of caveolae and caveolar FC at the cell surface (Fielding et al., 1997; Thyberg et al., 1998). In aortic endothelial cells, caveolin levels, already high, were not further increased by FC loading, but increased amounts of FC became caveola-associated (Zhu et al., 2000). A decrease in cellular FC was associated with a decrease in cell surface caveolae, and downregulation of caveolin synthesis (Hailstones et al., 1998). Caveolae were identified in primary fibroblasts and vascular smooth muscle cells as sites from which FC is preferentially transferred out of the cell (Fielding and Fielding, 1995; Fielding et al., 2002). It is not yet clear if this results directly from a destabilization of caveolar FC, for example following signal transduction, or if it involves the activity of caveolar ancillary proteins such as p-glycoprotein or SR-BI, to facilitate FC exchange between biological membranes (Liscovitch and Lavie, 2000; Liu et al., 2002). Together these data suggest a model for FC homeostasis in peripheral cells, in which a rise in cellular FC leads to an increase in both caveolin synthesis and the expression of cell surface caveolae. This in turn leads to an increase in FC efflux, and thus a decrease in cell FC, followed by downregulation of caveolin expression (Fielding et al., 1997).

Initiation of signal transduction from PDGF-R by PDGF is associated with a major, rapid decrease in the level of FC associated with caveolae. As much as 80% of initial FC content was lost within 5 min, at least in part via a transient 4-fold stimulation of FC efflux, when the physiological acceptor apolipoproptein A-1 (apo A-1) was present in the medium. Apo A-1 or lipid-poor prebeta-migrating HDL are the major acceptors of FC derived from caveolae. That this FC was directly derived from caveolae was shown by balance studies. The presence of apo A-1 (and stimulation of the loss of FC from caveolae) was associated with a 2–4 fold stimulation of protein kinase activity (Fielding et al., 2002). These data illustrate the dynamic relationship that exists between signal transduction and FC homeostasis in caveolae (Fig. 1). Whether other signals originating in caveolae are stimulated by FC efflux in a similar way remains to be determined.

The structure and regulation of raft-associated signal complexes have been determined mainly in blood leucocytes, where these make up the major proportion of FC-rich cell surface domains. A well-studied example is the T-cell immunoglobulin receptor. This signaling complex includes the proteins *lyn* and *lck*. Both are acylated, and the nonacylated proteins are inactive in signal transduction (Kovarova et al., 2001; Hawash et al., 2002; Lang et al., 2002). Raft integrity, and specifically the presence of FC, is also required. Unlike the case with caveolae, there is no evidence at present that the FC content of rafts is dynamically regulated. Their short lifetime probably limits such a role (Fig. 1).

To summarize, the structure, properties and functions of lipid rafts and caveolae, while significantly different, show some common features. The three-dimensional structure of caveolae permits the possibility of increased specificity in the efflux of FC, and amplification and dynamic control of signal transduction.

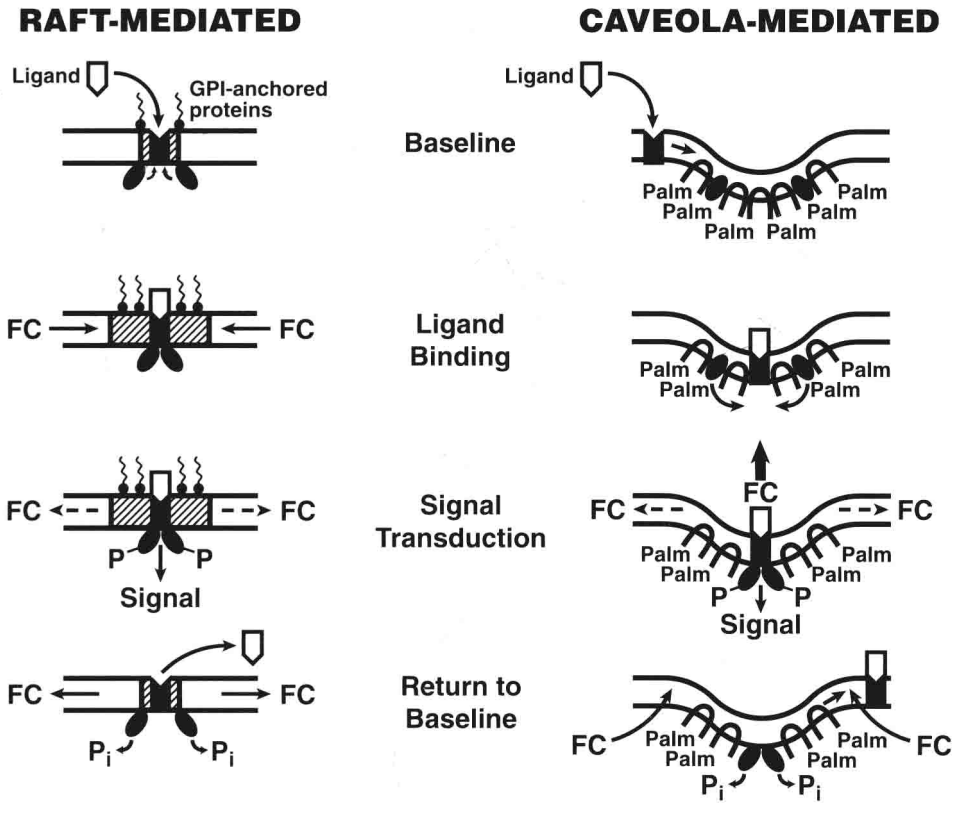

Fig. 1. Models for raft-mediated (left) and caveolae-mediated (right) signal transduction. The transient nature of raft complexes is compared with the semi-permanent existence of caveolae. The figure also shows the ability of caveolae to rapidly transfer FC to the extracellular space, in contrast to the lateral transfer in the plane of the membrane suggested for lipid rafts. The horseshoe shaped symbols represent caveolin; palm, palmitoylated caveolin.

3. Caveolae in cell division and aging

Cell division requires a doubling of cell-associated FC within a period of a few hours. In subconfluent primary skin fibroblasts, where the doubling time is 24–28 h, such an increase was complete within 8 h following S-phase, and preceded mitosis. This was achieved without any significant increase in cholesterogenesis above the minimal rates characteristic of quiescent cells (Fielding et al., 1999). The increase in cellular FC mass is the result both of an increase of the uptake of preformed lipoprotein FC, and a decrease in the rate of FC efflux. At the same time the expression of caveolae is reduced, and caveolin synthesis downregulated at the level of transcription. These effects are dependent on transcription factors E2F and p53. p53 acts as a tumor suppressor in many cell lines. Entry into E2F-dependent cell cycling depends on p53 downregulation, while p53 expression is associated with cell arrest. Chronic p53 overexpression leads to apoptosis.

The finding that the caveolin gene was p53-dependent (Fielding et al., 2000; Galbiati et al., 2001) may indicate that the downregulation of caveolae and caveolin expression represents a significant cell cycle regulatory pathway effective at the S/G2 interface, potentially involving both resistance to growth factors and retention of cellular FC.

In the aging cell, these relationships appear to be greatly modified. While the expression of p53 and caveolin are both increased, these changes were not associated with the expected increase in cell surface caveolae. High levels of total caveolin, but an absence of cell-surface caveolae, were reported in senescent human fibroblasts (Wheaton et al., 2001). These cells also lacked caveola-associated tyrosine kinases including the receptor proteins for several growth factors. In a second study, an increase in caveolin protein was found in aging cells, together with an increase in cytoplasmic vesicles that though identified as caveolae, obviously differed in structure and location from normal caveolae (Park et al., 2000). Instead, most caveolin in aging cells appeared to be present in intracellular vesicles. Here it could play no role in the stimulation of FC efflux or signaling via cell surface kinases. The aging cell is generally refractory to the effects of growth factors, probably at least in part associated with a reduction in the expression of caveolae. Together these data suggest that the induction of

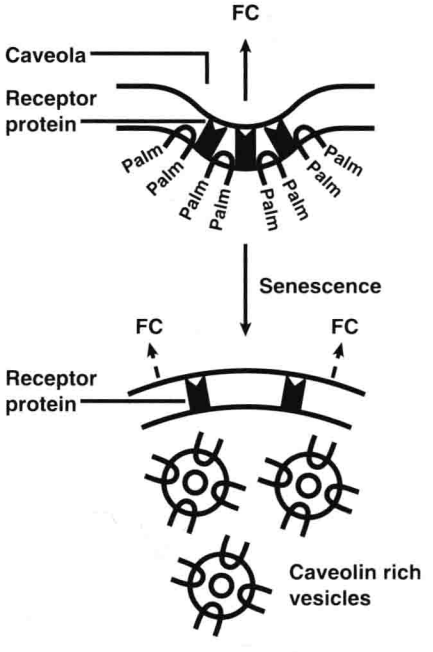

Fig. 2. Effects of aging/senescence on the distribution of caveolae and caveolin. The model shown suggests that the inability of senescent cells to palmitoylate caveolin may be central to the inability of caveolin to form cell surface caveolae, and the disruption of normal cell-surface tyrosine kinase-based signaling complexes.

caveolin expression is normal in aging cells, but that the conversion of this caveolin to caveolae functional at the cell surface is not.

Caveolin at the cell surface is palmitoylated at residues 133, 143 and 156. Early experiments did not distinguish between vesicles containing caveolin and cell surface caveolae, and reported that mutant, palmitoylation-defective caveolin was recovered, like wild-type caveolin, in the detergent-resistant membrane fraction (Dietzen et al., 1995) while observing that caveolin polymerization was stabilized by acylation (Monier et al., 1996). Functional studies of caveola-dependent signaling now show that in the absence of palmitoylation, normal caveolae are not formed (Uittenbogaard and Smart, 2000).

Oxidative stress is a significant factor in cellular aging, and stress induced in endothelial cells was associated with an inhibition of caveolin palmitoylation (Parat et al., 2002) and trafficking (Kang et al., 2000; Parat et al., 2002). This suggests that in aging cells, a decrease in cell surface caveolae, and the accumulation of intracellular caveolin vesicles mediated by oxidative stress, could be responsible for abnormalities in both signaling by receptor kinases, and potentially, in FC homeostasis (Fig. 2).

4. Summary

Several lines of investigation connect FC homeostasis and signaling via caveolae and lipid rafts. Caveolae appear to represent a stabilized, three-dimensional evolution of rafts, that can control their FC content dynamically, both during signal transduction and in response to lipid loading. In the normal dividing cell, this system limits signaling from the cell surface except to initiate the cell cycle, and retains FC as required for cell membrane synthesis, despite the presence of extracellular lipoprotein FC acceptors. In the aging cell, these mechanisms are deranged and while caveolin accumulates in intracellular vesicles, rather than in cell surface caveolae, and caveolin palmitoylation is inhibited. A detailed study of the relationship between palmitoylation status and caveolar function would be of great interest. Studies of the molecular basis of these effects should also offer many opportunities to understand the role of the plasma membrane in aging.

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