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Review

Is Alzheimer's Disease a Disorder of Mitochondria-Associated Membranes?

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Abstract. The subcellular localization of presenilin-1 (PS1) and presenilin-2 (PS2), two proteins that, when mutated, cause familial Alzheimer's disease (AD), is controversial. We have discovered that mitochondria-associated membranes (MAM) – a specialized subcompartment of the endoplasmic reticulum (ER) involved in lipid metabolism and calcium homeostasis that physically connects ER to mitochondria – is the predominant subcellular location for PS1 and PS2, and for γ-secretase activity. We hypothesize that presenilins play a role in maintaining MAM function, and that not only altered amyloid-β levels and hyperphosphorylated tau, but also many other features of AD (e.g., altered phospholipid and cholesterol metabolism, aberrant calcium homeostasis, and abnormal mitochondrial dynamics) result from compromised MAM function. The localization of presenilins and γ-secretase in MAM may help reconcile disparate ideas regarding the pathogenesis of AD, under a unifying hypothesis that could explain many features of both sporadic and familial AD, thereby taking AD research in a new and fruitful direction.

Keywords: Alzheimer's disease, calcium, cholesterol, endoplasmic reticulum (ER), mitochondria, mitochondria-associated membranes (MAM), phospholipids

ALZHEIMER'S DISEASE

Alzheimer's disease (AD), the most common late onset neurodegenerative dementing disorder, is characterized by progressive neuronal loss, especially in the hippocampus and cortex [1]. The two main histopathological hallmarks of AD are the accumulation of extracellular neuritic plaques, containing amyloid-β (Aβ), and of neurofibrillary tangles, consisting mainly of hyperphosphorylated forms of the microtubule-associated protein tau [1]. Most AD patients are sporadic (SAD), but three genes have been associated with the familial form (FAD): amyloid-β protein precursor (AβPP), presenilin-1 (PS1), and presenilin-2 (PS2). Clinically, FAD is similar to SAD but has earlier onset.

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Presenilins are components of the γ-secretase complex (also containing APH1, nicastrin, and PEN2) that, together with β-secretase, processes AβPP to produce Aβ [1]. In the mainstream view, both SAD and FAD arise when AβPP is processed to Aβ, which accumulates in extracellular plaques. Aβ is toxic to cells and the resulting stress promotes tau hyperphosphorylation, leading to the tangles. The overall process has been called the "amyloid cascade" hypothesis [2,3]. This hypothesis reconciles findings from different approaches to the disease and has served as the basis for many key experiments *in vivo* and *in vitro*. However, certain questions that are central to understanding the pathogenesis of AD and the processing of Aβ remain unsolved.

The first question concerns features of AD that are not obviously linked to plaque or tangle formation. While plaques and tangles are hallmarks of the disease, other apparently unrelated laboratory abnormali-

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ties are routinely detected in patients, including elevated cholesterol [4], altered fatty acid [5,6], glucose [7, 8], and phospholipid [9] metabolism, aberrant calcium homeostasis [10], and mitochondrial dysfunction [11]. These features of AD have received far less attention because of the lack of direct links to the amyloid cascade, and have engendered numerous competing hypotheses to explain the pathogenesis of AD. These include tau hyperphosphorylation [12], altered lipid [13], cholesterol [14], and glucose metabolism [15,16], aberrant calcium homeostasis [17], glutamate excitotoxicity [18], inflammation [18,19], ER stress and the unfolded protein response (UPR) [20–23], and mitochondrial dysfunction and oxidative stress [24]. It remains to be determined to what degree these phenomena are causally interlinked, and whether they may be direct outcomes of defects in AβPP processing.

The second issue is commonly referred to as the "spatial paradox" [25]: whereas PS1 is believed to be located mainly in the ER [26] and Golgi [26,27] as a component of an intracellular γ-secretase [28], processing of AβPP to release extracellular Aβ is believed to occur at or near the plasma membrane (PM) [29]. Thus, there is an apparent physical disconnect between the intracellular location of presenilins and γ-secretase activity on the one hand and the presumed site of Aβ synthesis on the other.

One potential solution to the spatial paradox would be to revise our current opinion on the subcellular distributions of presenilins. Indeed, although many believe that presenilins are located in the ER and/or Golgi, in truth, the subcellular localization of PS1 and PS2 has been the subject of controversy, with localizations reported in other compartments, including the nuclear envelope [30], endosomes [31], lysosomes [32], mitochondria [33], and the plasma membrane [28], where it is especially enriched at intercellular contacts known as adherens junctions [34].

Our understanding of the pathogenesis of AD, and by extension, our efforts to develop therapies for this devastating disorder, is highly dependent on a precise localization of this key proteolytic event and potential therapeutic target.

MITOCHONDRIA-ASSOCIATED MEMBRANES

Mitochondria-associated ER membranes (MAM) is a specialized subcompartment of the ER that connects this organelle to mitochondria, both biochemically and physically [35–38]. It plays a critical role in calcium homeostasis, as well as in various housekeeping functions, such as phospholipid, glucose, sphingolipid, ganglioside, cholesterol, and fatty acid metabolism [38], as well as in calcium homeostasis and signaling [38] and in apoptosis [39]. This physical connection has been proposed to be mediated by phosphofurin acidic cluster sorting protein 2 (PACS2) [39] and mitofusin 2 (MFN2) [40].

MAM is distinct from "bulk" ER in its composition and behavior. For example, MAM is enriched in neutral lipids and cholesterol [41,42], which, upon subcellular fractionation, allows it to be separated from bulk ER as a low-density fraction [43]. In fact, MAM has been described as a detergent-resistant microdomain of the ER having the properties of intracellular lipid rafts [42, 44].

A POTENTIAL ROLE FOR MAM IN THE PATHOGENESIS OF AD

We recently reported a novel subcellular localization for presenilins in MAM [45], and discuss here a mechanism that could link this localization to the known metabolic changes in AD. Furthermore, based on several lines of evidence, we propose that defects in MAM function play a key role in the pathogenesis of both the sporadic and familial forms of AD.

PS1 and PS2 are enriched in the MAM

As alluded to above, PS1 has been localized to almost every membranous compartment of the cell, including ER [26,30,46–48], the Golgi apparatus [26,27,46,49], endosomes [31,50], lysosomes [32], the nuclear envelope [30], mitochondria [33,51], and the plasma membrane [28,52–55], including adherens junctions [34, 56].

Using a combination of biochemical, immunocytochemical, and functional assays, we have shown that, apart from its presence in the plasma membrane, PS1 and PS2 are actually highly enriched in only one other compartment of the cell, namely MAM [45]. Moreover, besides identifying MAM as the main subcellular compartment for the presenilins, we also found that γ-secretase activity associated with AβPP processing is essentially confined to the MAM [45]. Thus, our identification of AβPP together with high levels of both presenilins and γ-secretase activity in the same subcel-

lular compartment (i.e., MAM) could help resolve the spatial paradox.

Our results were somewhat surprising, because in the 15 years since mutations in PS1 [57] and PS2 [58, 59] were identified, many laboratories, using both morphological and biochemical approaches, localized the presenilins to numerous subcellular compartments, but never specifically to MAM.

As we noted in our initial report [45], we believe that the discrepancy between our results and those of others was due mainly to the use of appropriate markers that could identify MAM unambiguously. Furthermore, given the lipid raft-like nature of MAM, it is not surprising that standard techniques used to isolate membranous organelles would unavoidably result in co-purification of MAM along with bulk ER, Golgi, endosomes, and mitochondria [49,60–63]. We also note that both AβPP [64] and Aβ [65,66] have been detected in mitochondria, but because of the contamination of most mitochondrial preparations with MAM, these polypeptides could also be MAM-associated proteins that were inadvertently assigned to mitochondria [67].

Moreover, the fact that MAM is a detergent-resistant membrane (DRM) microdomain [44] means that immunocytochemical techniques that rely on the use of detergents for permeabilization will only reveal epitopes that are not embedded in DRMs, as exemplified by the difficulty in identifying plasma membrane lipid raft proteins [68,69], where the use of detergents as permeabilization agents is limited [70–73].

In the case of the presenilins, we have found that the use of detergents, such as Triton X-100 (TX100), to permeabilize cells prior to the application of antibodies against PS1 or PS2 yields a dramatically different result as compared to that obtained following the use of organic solvents, such as methanol (MeOH), for cell permeabilization (Fig. 1). If TX100 is used for permeabilization, the anti-PS1 signal is spread diffusely among essentially all membranous compartments of the cell (Fig. 1A). However, when MeOH is used, the signal co-localizes predominantly with the MitoTracker Red signal, especially in the perinuclear region, where most of the ER resides (Fig. 1B). We confirmed these results by performing a triple staining in human fibroblasts permeabilized with MeOH, labeling mitochondria with MitoTracker Red (red) and immunostaining with anti-PS1 (green) and with anti-KDEL (blue) as an ER marker (Fig. 1C).

Our finding that presenilins and γ-secretase activity are enriched in MAM [45] is consistent with reports that PS1 and other γ-secretase components [31,74], as well as γ-secretase activity itself [75–80], are present in DRMs and/or lipid rafts.

Altered MAM function in AD

The presence of presenilins, γ-secretase activity, and AβPP in MAM is consistent with the amyloid cascade hypothesis. Nevertheless, we believe that the accumulation of plaques and tangles in AD, which are clearly detrimental, are merely features of the disease (and probably exacerbate its course and progression), but are not its underlying cause. Rather, we propose that the fundamental pathogenetic cause of AD is the alteration of MAM function, which could explain many of the disparate features of the disease noted above, including, but not limited to, the formation of plaques and tangles. Three aspects of MAM function are particularly noteworthy (Fig. 2A).

The role of MAM in lipid metabolism

More than fifteen proteins involved in fatty acid, phospholipid, glycolipid, and triglyceride metabolism are enriched in the MAM [38,45]. The role of MAM as a compartment connecting ER with mitochondria is illustrated very nicely by a major cellular pathway for the synthesis of phosphatidylethanolamine (PtdEtn). PtdEtn can be synthesized *de novo* in the ER from free ethanolamine via the Kennedy pathway [81], but most PtdEtn is made in a collaborative effort between MAM and mitochondria (see Fig. 2A): phosphatidylserine (PtdSer) is synthesized in the MAM via the serine-exchange enzyme phosphatidylserine synthase 2 (PTDSS2); PtdSer is then translocated to the mitochondria [82], where it is decarboxylated to PtdEtn by phosphatidylserine decarboxylase (PISD); finally PtdEtn is transferred back to the MAM, where it is methylated by phosphatidylethanolamine methyltransferase (PEMT) to generate phosphatidylcholine (PtdCho) [83].

The kinetics of trafficking of PtdSer from MAM to mitochondria is a recognized measure of MAM function [84]. Using such an approach, we recently detected a significant difference in PtdSer and PtdEtn synthesis in PS-mutant versus control cells (data not shown). This result is consistent with the reported aberrations in phospholipid profiles in AD patients [9,85]. Intriguingly, significant changes in PtdEtn and PtdCho levels have also been found in the brains of SAD patients [86], implying that a defect in this known MAM function could play a role not only in the familial, but also in the sporadic, form of the disease.

Remarkably, phospholipids, and especially PtdEtn, play a role in tau phosphorylation [87]. Thus, it is

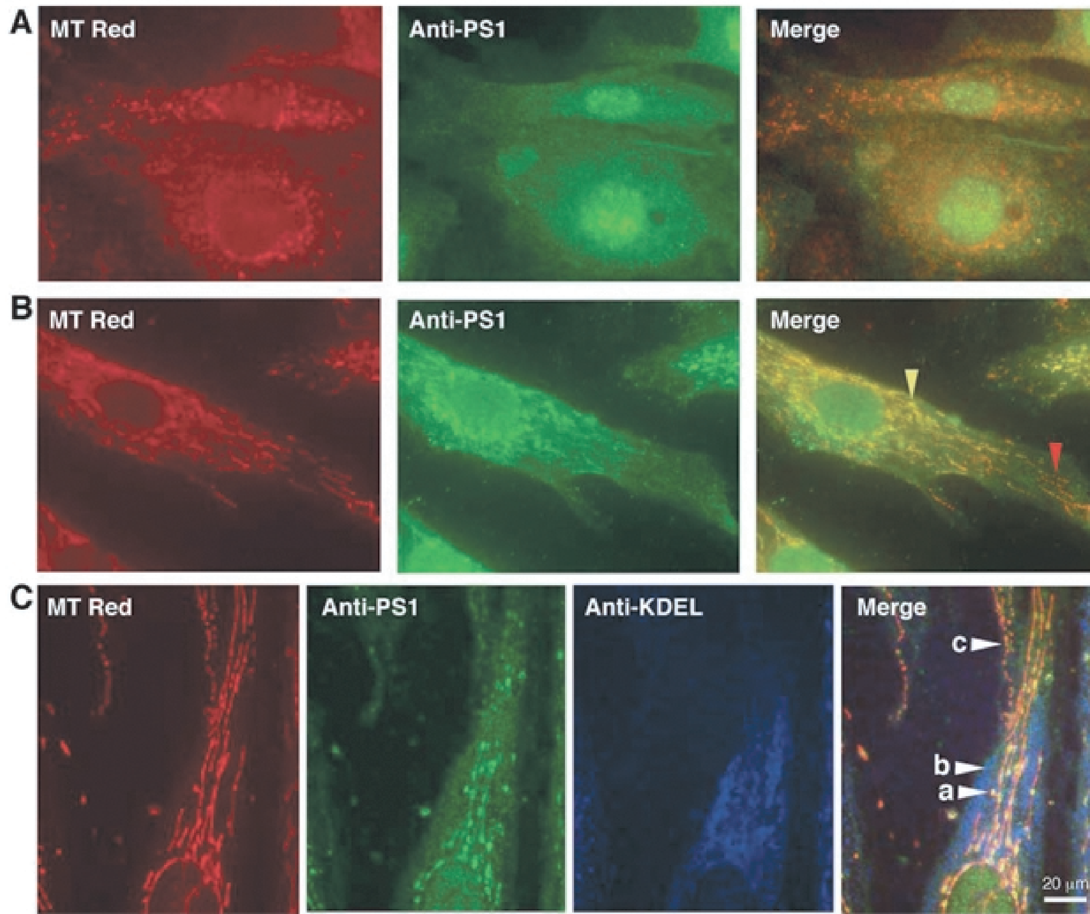

Fig. 1. Immunolocalization of PS1 in human fibroblasts. A) Fixation with paraformaldehyde (PF) and permeabilization with TX100. Note poor co-localization of the two signals (the orange staining in the merge panel is the non-specific overlap of the MitoTracker Red [MT Red] stain with the diffuse anti-PS1 stain). B) Fixation with PF and permeabilization with MeOH. Note the co-localization of PS1 and MT Red in the perinuclear region (yellow arrowhead) but not in more distal regions (red arrowhead). Images captured by confocal microscopy (100x). C) Co-localization of MT Red, anti-PS1, and anti-KDEL, following MeOH fixation. Note that PS1 and MT Red "co-localize" (i.e., yellow signal), but only in regions where both ER and mitochondria are present (arrowhead marked "a"), whereas yellow signals are barely detectable in regions that contain ER but no mitochondria (arrowhead marked "b") or mitochondria but no ER (arrowhead marked "c"). This result confirms that the yellow areas do not represent a true co-localization of ER and mitochondria, but rather a region below the level of confocal resolution where the two organelles are in apposition and make contact (i.e., MAM) (see also Fig. 3 in [45]).

possible that altered MAM function in AD patient cells could help explain the accumulation of tangles containing hyperphosphorylated forms of tau [88].

The role of MAM in cholesterol metabolism

MAM is not only a lipid raft-like domain rich in cholesterol, but it also contains a key enzyme of cholesterol metabolism, namely, acyl-CoA:cholesterol acyltransferase (ACAT) [89], which catalyzes the conversion of free cholesterol to cholesteryl esters [90]. There are two ACAT isoforms in mammals – ACAT1 (gene *SOAT1*) and ACAT2 (gene *SOAT2*) – but ACAT1 is the predominant isoform in humans (and is the main functioning isoform in mouse brain [91]), whereas ACAT2 is expressed mainly in the intestine [90]. ACAT1 affects cholesterol homeostasis by controlling the dynamic equilibrium between membrane-bound free cholesterol and cholesteryl esters stored in cytoplasmic lipid droplets [14].

The relationship of MAM-localized ACAT1 to AD is intriguing, for at least three reasons. First, ACAT1 affects AβPP processing [91–93] and is required for the generation of Aβ [14,94,95]. Second, there may be a positive feedback loop between MAM content and ACAT1 activity, because elevated cholesterol stimu-

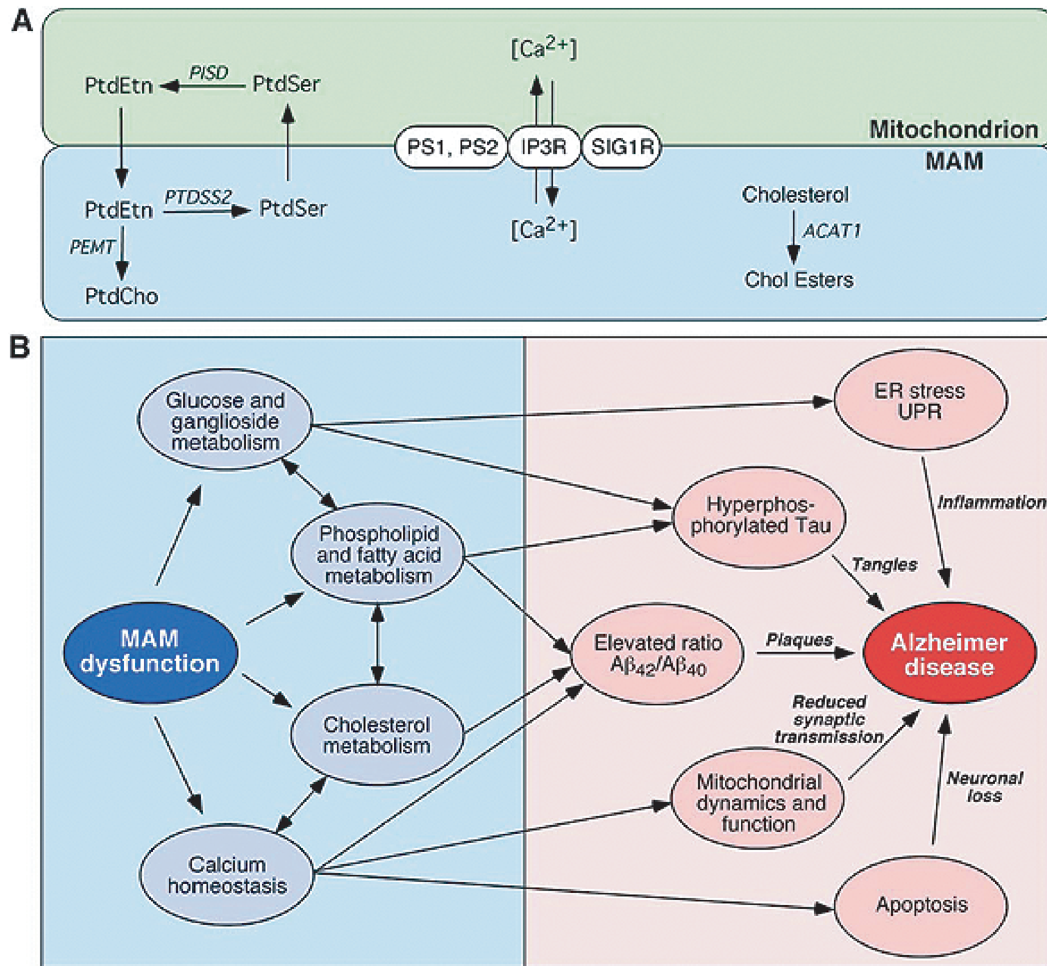

Fig. 2. Working model for the pathogenesis of AD due to abnormalities in MAM function. A) Illustrated are the three MAM functions discussed in the text: phospholipid transport and biosynthesis (pathway at left); calcium homeostasis (center); and cholesterol metabolism (right). Note that presenilins have been associated with IP3 receptors (IP3R), which in turn associate with the sigma-1 receptor (SIG1R); both are involved in Ca^{2+} signaling. B) Altered MAM function (resulting, for example, from mutations in PS1) affect the indicated pathway (light blue ovals), each of which can have one or more effects (pink ovals) that result in the features of AD (bold). The model as shown is somewhat simplified, as the various MAM functions can actually affect each other (double-headed arrows). UPR, unfolded protein response.

lates an increase in low density ER vesicles (presumably MAM) containing ACAT1 [96]. Finally, MAM is also enriched in microsomal triacyglycerol transfer protein large subunit (MTTP) [89] that, together with protein disulfide isomerase (PDI) [97], catalyzes the transport of cholesteryl esters, phospholipids, and triglycerides between phospholipid surfaces, and which is required for the secretion of plasma lipoproteins [97].

Importantly, patients with AD have elevated circulating cholesterol [4,98], and ACAT1 is elevated in the blood [99] and in fibroblasts [100] from AD patients, but the reason for these observations is currently unclear. We suggest that altered communication between the MAM and mitochondria in AD results in increased ACAT1 activity, thereby affecting circulating cholesterol levels and Aβ production.

The role of MAM in calcium signaling

Given that the ER is the cell's main source, and that the mitochondrion is the cell's main sink, of calcium [101], it is perhaps not surprising that MAM, the compartment connecting these two organelles, plays a key role in calcium trafficking [38]. Proteins involved in Ca^{2+} trafficking have been shown to be enriched in the MAM, including inositol-1,4,5-trisphosphate (IP3) receptors [102,103], ryanodine receptors [104,105], the sigma-1 opioid receptor [106], and perhaps even

calnexin [107], emphasizing the intimate relationship between ER and mitochondria in regulating calcium homeostasis [108]. Conversely, Ca^{2+} regulates the apposition of ER and mitochondria via autocrine motility factor receptor (AMFR) [109], an apparently MAM-enriched protein [110,111].

A defect in calcium signaling due to altered MAM function could explain the well-known disturbances in calcium homeostasis in AD [17,112,113], including enhanced IP3-mediated release of Ca^{2+} in fibroblasts from patients with SAD [114] and with FAD due to mutations in PS1 and PS2 [114–117], as well as in PS-mutant mouse cells [118].

Since MAM plays a role in calcium buffering [37], mutations in PS1 that alter MAM function could cause the defects in calcium homeostasis observed in AD patients. Consistent with this, presenilins are required for Ca^{2+} influx into cells through "store-operated" Ca^{2+} (SOC) channels located in the plasma membrane ("capacitative calcium entry" [CCE]), which require microdomains containing both an active sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) – a protein associated with PS1 [119,120] – and neighboring mitochondria [121]. In cells with FAD-linked mutations, ER [Ca^{2+}] was increased [117,122] and CCE was inhibited [122–124], with downstream effects on AβPP processing and Aβ production [123–125].

Perhaps the most compelling evidence for a role of MAM in calcium dysregulation in AD is the finding that both PS1 and PS2 interact directly with IP3 receptors (IP3R), and that FAD-linked mutations in both proteins not only enhance IP3R gating dramatically to increase intracellular Ca^{2+} but also stimulate Aβ production [126,127].

Among calcium's many functions is its role as a key regulator of mitochondrial movement along microtubules in response to changes in the local Ca^{2+} gradient [128]. Mitochondria with normal membrane potential tend to move towards the periphery, whereas loss of membrane potential and of ATP synthesis result in increased retrograde transport towards the cell body [129]. Two cargo adaptor proteins, Miro and Milton, are implicated in the specific linkage of mitochondria to microtubules (via kinesin-1) [130]. Disruption of both Miro [131] and Milton [132] function causes redistribution of mitochondria from terminals to the cell body. Importantly, Miro binds Ca^{2+} and, when mutated, causes aggregation of mitochondria in the perinuclear region [131]. Thus, Miro has the potential to be an important regulator of mitochondrial motility, in essence operating as a sensor of local concentrations of Ca^{2+} and ATP: in the Ca^{2+}-unbound state, Miro binds Milton and mitochondria are attached to microtubules, whereas in the Ca^{2+}-bound state, Miro cannot bind Milton and mitochondria are uncoupled from microtubules [128,133,134]. Remarkably, only Ca^{2+} mobilized via the IP3 receptors (or, in muscle, the ryanodine receptors) could effect this result [128].

Since mitochondria need to be positioned strategically at sites where metabolic demand is high [135,136], a problem in calcium-mediated mitochondrial movement along axons in AD could have highly deleterious effects on neuronal function in general and on synaptic transmission in particular. We have observed aberrant mitochondrial dynamics in fibroblasts from FAD patients (data not shown), and altered mitochondrial dynamics has also been observed in cells from patients with SAD, including defects in mitochondrial distribution, transport, and morphology [11,137–139]. It remains to be seen whether these changes are related to altered MAM function.

A WORKING MODEL FOR MAM-MEDIATED PATHOGENESIS OF AD

We think that the enrichment of presenilins in MAM is important conceptually to our understanding of AD, for at least three reasons. First, MAM is a true subcompartment with specific functions, not merely a topological elaboration of the ER. Second, MAM is a known key control point for cholesterol, glucose, lipid, and calcium metabolism [83], all of which functions are deranged in AD; we think that this is more than mere coincidence. Third, MAM connects ER to mitochondria, implying that mitochondria may play a much more important role in AD than had previously been suspected, as dysfunctional MAM could explain the aberrant mitochondrial dynamics and function found in the disease.

Accordingly, we propose a working model in which altered MAM function plays a fundamental role in the pathogenesis of AD, and provides a unifying hypothesis that could explain many of the seemingly-unrelated features of the disease (Fig. 2B). Specifically, we propose that altered MAM function could explain the elevated ratio of $A\beta_{42}/A\beta_{40}$ (via changes in the content of cholesterol), the hyperphosphorylation of tau (via changes in phospholipid metabolism), and the aberrant mitochondrial dynamics, with retention of large numbers of [fragmented] mitochondria in the perinuclear

region (via abnormal Ca^{2+} transport between ER and mitochondria).

Although we describe here a relatively simple one-to-one relationship between individual MAM functions and individual aspects of AD pathology, the reality is likely to be far more complicated, as various MAM components probably interact with, and affect, each other. For example, changes in [Ca^{2+}] almost certainly affect the activity of other MAM (and mitochondrial) proteins that are calcium-sensitive (e.g., PTDSS2, which is both Ca^{2+}- and ATP-sensitive [67,140]), with downstream effects not only on mitochondrial dynamics, but also on Aβ production and apoptosis [141]. Similarly, altered cholesterol metabolism affects not only Aβ production, but may also change the topology of the MAM membrane [142], a cholesterol-rich DRM, thereby affecting not just AβPP cleavage (e.g., by influencing the orientation of AβPP in the membrane and hence the position at which it is cleaved by the γ-secretase [142]), but also the amounts, activities, and interactions among other membrane-embedded MAM proteins.

Importantly, while aberrant MAM function can ultimately result in the deposition of Aβ in plaques and of hyperphosphorylated tau in tangles [88], we believe that these "canonical" features of AD are not the primary cause of the disease [3,143]. Rather, we propose that they are consequences of pre-existing metabolic disturbances in MAM. Put another way, we believe that it is overall MAM dysfunction, and not plaques or tangles *per se*, that are important in triggering the onset of the disease.

Notably, all of the metabolic and morphological phenotypes noted above have been found in both FAD and SAD, including, most recently, perinuclear mitochondria in SAD [11]. Thus, the "MAM hypothesis" offers a potentially coherent and unified explanation of many of the features of both the familial and sporadic forms of the disease. It also opens the door to new ways of thinking about treatment that are complementary to approaches focused directly on plaques and tangles. In fact, a strategy designed to "fix" the MAM may prove to be a more tractable therapeutic approach. In addition, we believe that it may be possible to exploit aberrant MAM function as a useful marker for the development of a diagnostic tool for AD.

While the MAM hypothesis will require modification in some of its particulars, we believe that the finding that presenilins are physically and functionally associated with MAM, and that AD patients display a number of defects associated with MAM function, is sufficiently compelling to warrant further investigation.

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REFERENCES

[1] Goedert M, Spillantini MG (2006) A century of Alzheimer's disease. *Science* **314**, 777-781.
[2] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356.
[3] Pimplikar SW (2009) Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *Int J Biochem Cell Biol* **41**, 1261-1268.
[4] Stefani M, Liguri G (2009) Cholesterol in Alzheimer's disease: unresolved questions. *Curr Alz Res* **6**, 15-29.
[5] Martín V, Fabelo N, Santpere G, Puig B, Marín R, Ferrer I, Díaz M (2010) Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex. *J Alzheimers Dis* **19**, 489-502.
[6] Fraser T, Tayler H, Love S (2010) Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. *Neurochem Res* **35**, 503-513.
[7] Hoyer S, Oesterreich K, Wagner O (1988) Glucose metabolism as the site of the primary abnormality in early-onset dementia of Alzheimer type? *J Neurol* **235**, 143-148.
[8] Liu F, Shi J, Tanimukai H, Gu J, Gu J, Grundke-Iqbal I, Iqbal K, Gong CX (2009) Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. *Brain* **132**, 1820-1832.
[9] Pettegrew JW, Panchalingam K, Hamilton RL, McClure RJ (2001) Brain membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res* **26**, 771-782.
[10] Bezprozvanny I, Mattson MP (2008) Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci* **31**, 454-463.
[11] Wang X, Su B, Zheng L, Perry G, Smith MA, Zhu X (2009) The role of abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease. *J Neurochem* **109**, 153-159.
[12] Takashima A, Murayama M, Murayama O, Kohno T, Honda T, Yasutake K, Nihonmatsu N, Mercken M, Yamaguchi H, Sugihara S, Wolozin B (1998) Presenilin 1 associates with glycogen synthase kinase-3β and its substrate tau. *Proc Natl Acad Sci U S A* **95**, 9637-9641.

[13] Jin JK, Kim NH, Lee YJ, Kim YS, Choi EK, Kozlowski PB, Park MH, Kim HS, Min do S (2006) Phospholipase D1 is up-regulated in the mitochondrial fraction from the brains of Alzheimer's disease patients. *Neurosci Lett* **407**, 263-267.

[14] Puglielli L, Tanzi RE, Kovacs DM (2003) Alzheimer's disease: the cholesterol connection. *Nature Neurosci* **6**, 345-351.

[15] Ibanez V, Pietrini P, Alexander GE, Furey ML, Teichberg D, Rajapakse JC, Rapoport SI, Schapiro MB, Horwitz B (1998) Regional glucose metabolic abnormalities are not the result of atrophy in Alzheimer's disease. *Neurology* **50**, 1585-1593.

[16] Gong CX, Liu F, Grundke-Iqbal I, Iqbal K (2006) Impaired brain glucose metabolism leads to Alzheimer neurofibrillary degeneration through a decrease in tau O-GlcNAcylation. *J Alzheimers Dis* **9**, 1-12.

[17] Smith IF, Green KN, LaFerla FM (2005) Calcium dysregulation in Alzheimer's disease: recent advances gained from genetically modified animals. *Cell Calcium* **38**, 427-437.

[18] Ringheim GE, Szczepanik AM (2006) Brain inflammation, cholesterol, and glutamate as interconnected participants in the pathology of Alzheimer's disease. *Curr Pharm Des* **12**, 719-738.

[19] Salminen A, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T (2009) Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence via pattern recognition receptors. *Prog Neurobiol* **87**, 181-194.

[20] Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P, Scheper W (2005) The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol* **110**, 165-172.

[21] Unterberger U, Hoftberger R, Gelpi E, Flicker H, Budka H, Voigtlander T (2006) Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases *in vivo*. *J Neuropathol Exp Neurol* **65**, 348-357.

[22] Salminen A, Kauppinen A, Suuronen T, Kaarniranta K, Ojala J (2009) ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. *J Neuroinflammation* **6**, 41-53.

[23] Costa RO, Ferreiro E, Cardoso SM, Oliveira CR, Pereira CM (2010) ER stress-mediated apoptotic pathway induced by amyloid-β. *J Alzheimers Dis* **20**, 625-636.

[24] Castellani R, Hirai K, Aliev G, Drew KL, Nunomura A, Takeda A, Cash AD, Obrenovich ME, Perry G, Smith MA (2002) Role of mitochondrial dysfunction in Alzheimer's disease. *J Neurosci Res* **70**, 357-360.

[25] Cupers P, Bentahir M, Craessaerts K, Orlans I, Vanderstichele H, Saftig P, De Strooper B, Annaert W (2001) The discrepancy between presenilin subcellular localization and γ-secretase processing of amyloid precursor protein. *J Cell Biol* **154**, 731-740.

[26] De Strooper B, Beullens M, Contreras B, Levesque L, Craessaerts K, Cordell B, Moechars D, Bollen M, Fraser P, St George-Hyslop P, Van Leuven F (1997) Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins. *J Biol Chem* **272**, 3590-3598.

[27] Siman R, Velji J (2003) Localization of presenilin-nicastrin complexes and γ-secretase activity to the trans-Golgi network. *J Neurochem* **84**, 1143-1153.

[28] Tarassishin L, Yin YI, Bassit B, Li YM (2004) Processing of Notch and amyloid precursor protein by γ-secretase is spatially distinct. *Proc Natl Acad. Sci U S A* **101**, 17050-17055.

[29] Chyung JH, Raper DM, Selkoe DJ (2005) γ-Secretase exists on the plasma membrane as an intact complex that accepts substrates and effects intramembrane cleavage. *J Biol Chem* **280**, 4383-4392.

[30] Kimura N, Nakamura SI, Honda T, Takashima A, Nakayama H, Ono F, Sakakibara I, Doi K, Kawamura S, Yoshikawa Y (2001) Age-related changes in the localization of presenilin-1 in cynomolgus monkey brain. *Brain Res* **922**, 30-41.

[31] Vetrivel KS, Cheng H, Lin W, Sakurai T, Li T, Nukina N, Wong PC, Xu H, Thinakaran G (2004) Association of γ-secretase with lipid rafts in post-Golgi and endosome membranes. *J Biol Chem* **279**, 44945-44954.

[32] Pasternak SH, Bagshaw RD, Guiral M, Zhang S, Ackerley CA, Pak BJ, Callahan JW, Mahuran DJ (2003) Presenilin-1, nicastrin, amyloid precursor protein, and γ-secretase activity are co-localized in the lysosomal membrane. *J Biol Chem* **278**, 26687-26694.

[33] Ankarcrona M, Hultenby K (2002) Presenilin-1 is located in rat mitochondria. *Biochem Biophys Res Commun* **295**, 766-770.

[34] Marambaud P, Shioi J, Serban G, Georgakopoulos A, Sarner S, Nagy V, Baki L, Wen P, Efthimiopoulos S, Shao Z, Wisniewski T, Robakis NK (2002) A presenilin-1/γ-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. *EMBO J* **21**, 1948-1956.

[35] Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* **280**, 1763-1766.

[36] Levine T, Rabouille C (2005) Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. *Curr Opin Cell Biol* **17**, 362-368.

[37] Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G (2006) Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol* **174**, 915-921.

[38] Hayashi T, Rizzuto R, Hajnoczky G, Su TP (2009) MAM: more than just a housekeeper. *Trends Cell Biol* **19**, 81-88.

[39] Simmen T, Aslan JE, Blagoveshchenskaya AD, Thomas L, Wan L, Xiang Y, Feliciangeli SF, Hung CH, Crump CM, Thomas G (2005) PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. *EMBO J* **24**, 717-729.

[40] de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456**, 605-610.

[41] Hayashi T, Su TP (2003) Intracellular dynamics of sigma-1 receptors (σ1 binding sites) in NG108-15 cells. *J Pharmacol Exp Ther* **306**, 726-733.

[42] Hayashi T, Su TP (2003) Sigma-1 receptors (σ1 binding sites) form raft-like microdomains and target lipid droplets on the endoplasmic reticulum: roles in endoplasmic reticulum lipid compartmentalization and export. *J Pharmacol Exp Ther* **306**, 718-725.

[43] Vance JE (1990) Phospholipid synthesis in a membrane fraction associated with mitochondria. *J Biol Chem* **265**, 7248-7256.

[44] Hayashi T, Fujimoto M (2010) Detergent-resistant microdomains determine the localization of sigma-1 receptors to the endoplasmic reticulum-mitochondria junction. *Mol Pharmacol* **77**, 517-528.

[45] Area-Gomez E, de Groof AJ, Boldogh I, Bird TD, Gibson GE, Koehler CM, Yu WH, Duff KE, Yaffe MP, Pon LA, Schon EA (2009) Presenilins are enriched in endoplasmic

reticulum membranes associated with mitochondria. *Am J Pathol* **175**, 1810-1816.

[46] Walter J, Capell A, Grunberg J, Pesold B, Schindzielorz A, Prior R, Podlisny MB, Fraser P, Hyslop PS, Selkoe DJ, Hass C (1996) The Alzheimer's disease-associated presenilins are differentially phosphorylated proteins located predominantly within the endoplasmic reticulum. *Mol Med* **2**, 673-691.

[47] Capell A, Saffrich R, Olivo JC, Meyn L, Walter J, Grunberg J, Mathews P, Nixon R, Dotti C, Haass C (1997) Cellular expression and proteolytic processing of presenilin proteins is developmentally regulated during neuronal differentiation. *J Neurochem* **69**, 2432-2440.

[48] Busciglio J, Hartmann H, Lorenzo A, Wong C, Baumann K, Sommer B, Staufenbiel M, Yankner BA (1997) Neuronal localization of presenilin-1 and association with amyloid plaques and neurofibrillary tangles in Alzheimer's disease. *J Neurosci* **17**, 5101-5107.

[49] Annaert WG, Levesque L, Craessaerts K, Dierinck I, Snellings G, Westaway D, St George-Hyslop P, Cordell B, Fraser P, De Strooper B (1999) Presenilin 1 controls γ-secretase processing of amyloid precursor protein in pre-Golgi compartments of hippocampal neurons. *J Cell Biol* **147**, 277-294.

[50] Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R, Hartmann T (2002) Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci* **22**, 1679-1689.

[51] Hansson EM, Stromberg K, Bergstedt S, Yu G, Naslund J, Lundkvist J, Lendahl U (2005) Aph-1 interacts at the cell surface with proteins in the active γ-secretase complex and membrane-tethered Notch. *J Neurochem* **92**, 1010-1020.

[52] Schwarzman AL, Singh N, Tsiper M, Gregori L, Dranovsky A, Vitek MP, Glabe CG, St George-Hyslop PH, Goldgaber D (1999) Endogenous presenilin 1 redistributes to the surface of lamellipodia upon adhesion of Jurkat cells to a collagen matrix. *Proc Natl Acad Sci U S A* **96**, 7932-7937.

[53] Singh N, Talalayeva Y, Tsiper M, Romanov V, Dranovsky A, Colflesh D, Rudamen G, Vitek MP, Shen J, Yang X, Goldgaber D, Schwarzman AL (2001) The role of Alzheimer's disease-related presenilin 1 in intercellular adhesion. *Exp Cell Res* **263**, 1-13.

[54] Baki L, Marambaud P, Efthimiopoulos S, Georgakopoulos A, Wen P, Cui W, Shioi J, Koo E, Ozawa M, Friedrich VL, Jr., Robakis NK (2001) Presenilin-1 binds cytoplasmic epithelial cadherin, inhibits cadherin/p120 association, and regulates stability and function of the cadherin/catenin adhesion complex. *Proc Natl Acad Sci U S A* **98**, 2381-2386.

[55] Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Siman R, Robakis NK (2003) A CBP binding transcriptional repressor produced by the PS1/ε-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell* **114**, 635-645.

[56] Georgakopoulos A, Marambaud P, Efthimiopoulos S, Shioi J, Cui W, Li HC, Schutte M, Gordon R, Holstein GR, Martinelli G, Mehta P, Friedrich VL, Jr., Robakis NK (1999) Presenilin-1 forms complexes with the cadherin/catenin cell-cell adhesion system and is recruited to intercellular and synaptic contacts. *Mol Cell* **4**, 893-902.

[57] Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754-760.

[58] Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973-977.

[59] Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, Chi H, Lin C, Holman K, Tsuda T (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376**, 775-778.

[60] Sawamura N, Morishima-Kawashima M, Waki H, Kobayashi K, Kuramochi T, Frosch MP, Ding K, Ito M, Kim TW, Tanzi RE, Oyama F, Tabira T, Ando S, Ihara Y (2000) Mutant presenilin 2 transgenic mice. A large increase in the levels of Aβ42 is presumably associated with the low density membrane domain that contains decreased levels of glycerophospholipids and sphingomyelin. *J Biol Chem* **275**, 27901-27908.

[61] Kim SH, Lah JJ, Thinakaran G, Levey A, Sisodia SS (2000) Subcellular localization of presenilins: association with a unique membrane pool in cultured cells. *Neurobiol Dis* **7**, 99-117.

[62] Hansson CA, Frykman S, Farmery MR, Tjernberg LO, Nilsberth C, Pursglove SE, Ito A, Winblad B, Cowburn RF, Thyberg J, Ankarcrona M (2004) Nicastrin, presenilin, APH-1, and PEN-2 form active γ-secretase complexes in mitochondria. *J Biol Chem* **279**, 51654-51660.

[63] Grimm MO, Tschape JA, Grimm HS, Zinser EG, Hartmann T (2006) Altered membrane fluidity and lipid raft composition in presenilin-deficient cells. *Acta Neurol Scand Suppl* **114**, 27-32.

[64] Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK (2006) Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci* **26**, 9057-9068.

[65] Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, Trinchese F, Liu S, Gunn-Moore F, Lue LF, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, Wu H (2004) ABAD directly links Aβ to mitochondrial toxicity in Alzheimer's disease. *Science* **304**, 448-452.

[66] Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH (2006) Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum Mol Genet* **15**, 1437-1449.

[67] Baranska J (1989) Mechanism of the ATP-dependent phosphatidylserine synthesis in liver subcellular fractions. *FEBS Lett* **256**, 33-37.

[68] Foster LJ, De Hoog CL, Mann M (2003) Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proc Natl Acad Sci U S A* **100**, 5813-5818.

[69] Pike LJ (2009) The challenge of lipid rafts. *J Lipid Res* **50**, S323-S328.

[70] Melan MA, Sluder G (1992) Redistribution and differential extraction of soluble proteins in permeabilized cultured cells. Implications for immunofluorescence microscopy. *J Cell Sci* **101**, 731-743.

[71] Heerklotz H, Szadkowska H, Anderson T, Seelig J (2003) The sensitivity of lipid domains to small perturbations demonstrated by the effect of Triton. *J Mol Biol* **329**, 793-799.

[72] Heffer-Lauc M, Lauc G, Nimrichter L, Fromholt SE, Schnaar RL (2005) Membrane redistribution of gangliosides and glycosylphosphatidylinositol-anchored proteins in brain tissue sections under conditions of lipid raft isolation. *Biochim Biophys Acta* **1686**, 200-208.

[73] Heffer-Lauc M, Viljetic B, Vajn K, Schnaar RL, Lauc G (2007) Effects of detergents on the redistribution of gangliosides and GPI-anchored proteins in brain tissue sections. *J Histochem Cytochem* **55**, 805-812.

[74] Vetrivel KS, Cheng H, Kim SH, Chen Y, Barnes NY, Parent AT, Sisodia SS, Thinakaran G (2005) Spatial segregation of γ-secretase and substrates in distinct membrane domains. *J Biol Chem* **280**, 25892-25900.

[75] Wahrle S, Das P, Nyborg AC, McLendon C, Shoji M, Kawarabayashi T, Younkin LH, Younkin SG, Golde TE (2002) Cholesterol-dependent γ-secretase activity in buoyant cholesterol-rich membrane microdomains. *Neurobiol Dis* **9**, 11-23.

[76] Wada S, Morishima-Kawashima M, Qi Y, Misono H, Shimada Y, Ohno-Iwashita Y, Ihara Y (2003) γ-Secretase activity is present in rafts but is not cholesterol-dependent. *Biochemistry* **42**, 13977-13986.

[77] Urano Y, Hayashi I, Isoo N, Reid PC, Shibasaki Y, Noguchi N, Tomita T, Iwatsubo T, Hamakubo T, Kodama T (2005) Association of active γ-secretase complex with lipid rafts. *J Lipid Res* **46**, 904-912.

[78] Osawa S, Funamoto S, Nobuhara M, Wada-Kakuda S, Shimojo M, Yagishita S, Ihara Y (2008) Phosphoinositides suppress γ-secretase in both the detergent-soluble and -insoluble states. *J Biol Chem* **283**, 19283-19292.

[79] Hur JY, Welander H, Behbahani H, Aoki M, Franberg J, Winblad B, Frykman S, Tjernberg LO (2008) Active γ-secretase is localized to detergent-resistant membranes in human brain. *FEBS J* **275**, 1174-1187.

[80] Wakabayashi T, Craessaerts K, Bammens L, Bentahir M, Borgions F, Herdewijn P, Staes A, Timmerman E, Vandekerckhove J, Rubinstein E, Boucheix C, Gevaert K, De Strooper B (2009) Analysis of the γ-secretase interactome and validation of its association with tetraspanin-enriched microdomains. *Nat Cell Biol* **11**, 1340-1346.

[81] Voelker DR (2000) Interorganelle transport of aminoglycerophospholipids. *Biochim Biophys Acta* **1486**, 97-107.

[82] Voelker DR (1989) Phosphatidylserine translocation to the mitochondrion is an ATP-dependent process in permeabilized animal cells. *Proc Natl Acad Sci U S A* **86**, 9921-9925.

[83] Vance JE (2003) Molecular and cell biology of phosphatidylserine and phosphatidylethanolamine metabolism. *Prog Nucl Acid Res Mol Biol* **75**, 69-111.

[84] Schumacher MM, Choi JY, Voelker DR (2002) Phosphatidylserine transport to the mitochondria is regulated by ubiquitination. *J Biol Chem* **277**, 51033-51042.

[85] Zhou S, Zhou H, Walian PJ, Jap BK (2007) Regulation of γ-secretase activity in Alzheimer's disease. *Biochemistry* **46**, 2553-2563.

[86] Nitsch RM, Blusztajn JK, Pittas AG, Slack BE, Growdon JH, Wurtman RJ (1992) Evidence for a membrane defect in Alzheimer disease brain. *Proc Natl Acad Sci U S A* **89**, 1671-1675.

[87] Baudier J, Cole RD (1987) Phosphorylation of tau proteins to a state like that in Alzheimer's brain is catalyzed by a calcium/calmodulin-dependent kinase and modulated by phospholipids. *J Biol Chem* **262**, 17577-17583.

[88] Perreault S, Bousquet O, Lauzon M, Paiement J, Leclerc N (2009) Increased association between rough endoplasmic reticulum membranes and mitochondria in transgenic mice that express P301L tau. *J Neuropathol Exp Neurol* **68**, 503-514.

[89] Rusinol AE, Cui Z, Chen MH, Vance JE (1994) A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. *J Biol Chem* **269**, 27494-27502.

[90] Chang TY, Li BL, Chang CC, Urano Y (2009) Acyl-coenzyme A:cholesterol acyltransferases. *Am J Physiol Endocrinol Metab* **297**, E1-E9.

[91] Bryleva EY, Rogers MA, Chang CC, Buen F, Harris BT, Rousselet E, Seidah NG, Oddo S, LaFerla FM, Spencer TA, Hickey WF, Chang TY (2010) ACAT1 gene ablation increases 24(S)-hydroxycholesterol content in the brain and ameliorates amyloid pathology in mice with AD. *Proc Natl Acad Sci U S A* **107**, 3081-3086.

[92] Huttunen HJ, Peach C, Bhattacharyya R, Barren C, Pettingell W, Hutter-Paier B, Windisch M, Berezovska O, Kovacs DM (2009) Inhibition of acyl-coenzyme A: cholesterol acyl transferase modulates amyloid precursor protein trafficking in the early secretory pathway. *FASEB J* **23**, 3819-3828.

[93] Huttunen HJ, Puglielli L, Ellis BC, MacKenzie Ingano LA, Kovacs DM (2009) Novel N-terminal cleavage of APP precludes Aβ generation in ACAT-defective AC29 cells. *J Mol Neurosci* **37**, 6-15.

[94] Puglielli L, Konopka G, Pack-Chung E, Ingano LA, Berezovska O, Hyman BT, Chang TY, Tanzi RE, Kovacs DM (2001) Acyl-coenzyme A:cholesterol acyltransferase modulates the generation of the amyloid β-peptide. *Nat Cell Biol* **3**, 905-912.

[95] Puglielli L, Ellis BC, Ingano LA, Kovacs DM (2004) Role of acyl-coenzyme A:cholesterol acyltransferase activity in the processing of the amyloid precursor protein. *J Mol Neurosci* **24**, 93-96.

[96] Sakashita N, Chang CCY, Lei X, Fujiwara Y, Takeya M, Chang T-Y (2010) Cholesterol loading in macrophages stimulates formation of ER-derived vesicles with elevated ACAT1 activity. *J Lipid Res* **51**, 1263-1272.

[97] Wetterau JR, Combs KA, Spinner SN, Joiner BJ (1990) Protein disulfide isomerase is a component of the microsomal triglyceride transfer protein complex. *J Biol Chem* **265**, 9800-9807.

[98] Martins IJ, Berger T, Sharman MJ, Verdile G, Fuller SJ, Martins RN (2009) Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. *J Neurochem* **111**, 1275-1308.

[99] Pani A, Mandas A, Diaz G, Abete C, Cocco PL, Angius F, Brundu A, Mucaka N, Pais ME, Saba A, Barberini L, Zaru C, Palmas M, Putzu PF, Mocali A, Paoletti F, La Colla P, Dessi S (2009) Accumulation of neutral lipids in peripheral blood mononuclear cells as a distinctive trait of Alzheimer patients and asymptomatic subjects at risk of disease. *BMC Med* **7**, 66-77.

[100] Pani A, Dessì S, Diaz G, La Colla P, Abete C, Mulas C, Angius F, Cannas MD, Orru CD, Cocco PL, Mandas A, Putzu P, Laurenzana A, Cellai C, Costanza AM, Bavazzano A, Mocali A, Paoletti F (2009) Altered cholesterol ester cycle in skin fibroblasts from patients with Alzheimer's disease. *J Alzheimers Dis* **18**, 829-841.

[101] Berridge MJ (2002) The endoplasmic reticulum: a multifunctional signaling organelle. *Cell Calcium* **32**, 235-249.

[102] Mendes CC, Gomes DA, Thompson M, Souto NC, Goes TS, Goes AM, Rodrigues MA, Gomez MV, Nathanson MH, Leite MF (2005) The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca^{2+} signals into mitochondria. *J Biol Chem* **280**, 40892-40900.

[103] Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla T, Rizzuto R (2006)

Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. *J Cell Biol* **175**, 901-911.

[104] Kopach O, Kruglikov I, Pivneva T, Voitenko N, Fedirko N (2007) Functional coupling between ryanodine receptors, mitochondria and Ca^{2+} ATPases in rat submandibular acinar cells. *Cell Calcium* **43**, 469-481.

[105] Garcia-Perez C, Hajnoczky G, Csordas G (2008) Physical coupling supports the local Ca^{2+} transfer between sarcoplasmic reticulum subdomains and the mitochondria in heart muscle. *J Biol Chem* **283**, 32771-32780.

[106] Hayashi T, Su TP (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signaling and cell survival. *Cell* **131**, 596-610.

[107] Myhill N, Lynes EM, Nanji JA, Blagoveshchenskaya AD, Fei H, Simmen KC, Cooper TJ, Thomas G, Simmen T (2008) The subcellular distribution of calnexin is mediated by PACS-2. *Mol Biol Cell* **19**, 2777-2788.

[108] Mironov SL, Symonchuk N (2006) ER vesicles and mitochondria move and communicate at synapses. *J Cell Sci* **119**, 4926-4934.

[109] Wang HJ, Guay G, Pogan L, Sauve R, Nabi IR (2000) Calcium regulates the association between mitochondria and a smooth subdomain of the endoplasmic reticulum. *J Cell Biol* **150**, 1489-1498.

[110] Registre M, Goetz JG, St Pierre P, Pang H, Lagace M, Bouvier M, Le PU, Nabi IR (2004) The gene product of the gp78/AMFR ubiquitin E3 ligase cDNA is selectively recognized by the 3F3A antibody within a subdomain of the endoplasmic reticulum. *Biochem Biophys Res Commun* **320**, 1316-1322.

[111] Goetz JG, Nabi IR (2006) Interaction of the smooth endoplasmic reticulum and mitochondria. *Biochem Soc Trans* **34**, 370-373.

[112] Yu JT, Chang RC, Tan L (2009) Calcium dysregulation in Alzheimer's disease: from mechanisms to therapeutic opportunities. *Prog Neurobiol* **89**, 240-255.

[113] Small DH (2009) Dysregulation of calcium homeostasis in Alzheimer's disease. *Neurochem Res* **34**, 1824–1829.

[114] Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL (1994) Internal Ca^{2+} mobilization is altered in fibroblasts from patients with Alzheimer disease. *Proc Natl Acad Sci U S A* **91**, 534-538.

[115] Leissring MA, Paul BA, Parker I, Cotman CW, LaFerla FM (1999) Alzheimer's presenilin-1 mutation potentiates inositol 1,4,5-trisphosphate-mediated calcium signaling in *Xenopus* oocytes. *J Neurochem* **72**, 1061-1068.

[116] Leissring MA, Parker I, LaFerla FM (1999) Presenilin-2 mutations modulate amplitude and kinetics of inositol 1, 4,5-trisphosphate-mediated calcium signals. *J Biol Chem* **274**, 32535-32538.

[117] Nelson O, Tu H, Lei T, Bentahir M, de Strooper B, Bezprozvanny I (2007) Familial Alzheimer disease-linked mutations specifically disrupt Ca^{2+} leak function of presenilin 1. *J Clin Invest* **117**, 1230-1239.

[118] Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee SF, Hao YH, Serneels L, De Strooper B, Yu G, Bezprozvanny I (2006) Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* **126**, 981-993.

[119] Green KN, Demuro A, Akbari Y, Hitt BD, Smith IF, Parker I, LaFerla FM (2008) SERCA pump activity is physiologically regulated by presenilin and regulates amyloid β production. *J Cell Biol* **181**, 1107-1116.

[120] Jin H, Sanjo N, Uchihara T, Watabe K, St George-Hyslop P, Fraser PE, Mizusawa H (2010) Presenilin-1 holoprotein is an interacting partner of sarco endoplasmic reticulum calcium-ATPase and confers resistance to endoplasmic reticulum stress. *J Alzheimers Dis* **20**, 261-273.

[121] Jousset H, Frieden M, Demaurex N (2007) STIM1 knockdown reveals that store-operated Ca^{2+} channels located close to sarco/endoplasmic Ca^{2+} ATPases (SERCA) pumps silently refill the endoplasmic reticulum. *J Biol Chem* **282**, 11456-11464.

[122] Leissring MA, Akbari Y, Fanger CM, Cahalan MD, Mattson MP, LaFerla FM (2000) Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J Cell Biol* **149**, 793-798.

[123] Yoo AS, Cheng I, Chung S, Grenfell TZ, Lee H, Pack-Chung E, Handler M, Shen J, Xia W, Tesco G, Saunders AJ, Ding K, Frosch MP, Tanzi RE, Kim TW (2000) Presenilin-mediated modulation of capacitative calcium entry. *Neuron* **27**, 561-572.

[124] Leissring MA, Murphy MP, Mead TR, Akbari Y, Sugarman MC, Jannatipour M, Anliker B, Muller U, Saftig P, De Strooper B, Wolfe MS, Golde TE, LaFerla FM (2002) A physiologic signaling role for the γ-secretase-derived intracellular fragment of APP. *Proc Natl Acad Sci U S A* **99**, 4697-4702.

[125] LaFerla FM (2002) Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nature Rev Neurosci* **3**, 862-872.

[126] Cheung KH, Shineman D, Muller M, Cardenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, Foskett JK (2008) Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of $InsP_3$ receptor channel gating. *Neuron* **58**, 871-883.

[127] Cheung KH, Mei L, Mak DO, Hayashi I, Iwatsubo T, Kang DE, Foskett JK (2010) Gain-of-function enhancement of IP3 receptor modal gating by familial Alzheimer's disease-linked presenilin mutants in human cells and mouse neurons. *Sci Signal* **3**, ra22.

[128] Yi M, Weaver D, Hajnoczky G (2004) Control of mitochondrial motility and distribution by the calcium signal: a homeostatic circuit. *J Cell Biol* **167**, 661-672.

[129] Miller KE, Sheetz MP (2004) Axonal mitochondrial transport and potential are correlated. *J Cell Sci* **117**, 2791-2804.

[130] Liu X, Hajnoczky G (2009) Ca^{2+}-dependent regulation of mitochondrial dynamics by the Miro-Milton complex. *Int J Biochem Cell Biol* **41**, 1972-1976.

[131] Fransson S, Ruusala A, Aspenstrom P (2006) The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem Biophys Res Commun* **344**, 500-510.

[132] Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL (2002) Axonal transport of mitochondria to synapses depends on milton, a novel *Drosophila* protein. *Neuron* **36**, 1063-1077.

[133] Rice SE, Gelfand VI (2006) Paradigm lost: milton connects kinesin heavy chain to miro on mitochondria. *J Cell Biol* **173**, 459-461.

[134] Glater EE, Megeath LJ, Stowers RS, Schwarz TL (2006) Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. *J Cell Biol* **173**, 545-557.

[135] Li Z, Okamoto K, Hayashi Y, Sheng M (2004) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* **119**, 873-887.

[136] Zenisek D, Matthews G (2000) The role of mitochondria in presynaptic calcium handling at a ribbon synapse. *Neuron* **25**, 229-237.

[137] Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, Masliah E, Williams DS, Goldstein LS (2005) Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* **307**, 1282-1288.

[138] Wang X, Su B, Fujioka H, Zhu X (2008) Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. *Am J Pathol* **173**, 470-482.

[139] Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X (2009) Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci* **29**, 9090-9103.

[140] Voelker DR (1990) Characterization of phosphatidylserine synthesis and translocation in permeabilized animal cells. *J Biol Chem* **265**, 14340-14346.

[141] Wang HQ, Nakaya Y, Du Z, Yamane T, Shirane M, Kudo T, Takeda M, Takebayashi K, Noda Y, Nakayama KI, Nishimura M (2005) Interaction of presenilins with FKBP38 promotes apoptosis by reducing mitochondrial Bcl-2. *Hum Mol Genet* **14**, 1889-1902.

[142] Wang W, Yang L, Huang HW (2007) Evidence of cholesterol accumulated in high curvature regions: implication to the curvature elastic energy for lipid mixtures. *Biophys J* **92**, 2819-2830.

[143] Robakis N (2010) Are Aβ and its derivatives causative agents or innocent bystanders in AD? *Neurodegener Dis* **7**, 32-37.